Coagulation Factor IX (Recombinant), GlycoPEGylated N9-GP (nonacog beta pegol)

Control and prevention of bleeding episodes, perioperative management and routine prophylaxis in adults and children with hemophilia B

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Briefing Document

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Abbreviations

ABR	annualized bleeding rate
AsBR	annualized spontaneous bleeding rate
AE	adverse event
AMD	age-related macular degeneration
aPTT	activated partial thromboplastin time
AUC	area under the curve
BLA	Biologics license application
BMI	body mass index
BU	Bethesda unit
C_{30min}	FIX activity 30 min post dosing
CHO	Chinese hamster ovary
CI	confidence interval
CL	clearance
ECG	electrocardiogram
ED	exposure day
eGFR	estimated glomerular filtration rate
EMA	European Medicines Agency
EUHASS	European Haemophilia Safety Surveillance
EQ-5D	EuroQol
FDA	Food and Drug Administration
FIX	coagulation factor IX
FIXa	activated coagulation factor IX
FVIIIa	activated coagulation factor VIII
FXa	activated coagulation factor X
FXIII	coagulation factor XIII
Haem-A-QoL	Haemophilia quality of life questionnaire for adults and adolescents
Haemo-QoL	Haemophilia quality of life questionnaire for children
^{1}H-NMR	nuclear magnetic resonance
HCP	host cell protein
HED	human equivalent dose
HRQoL	health-related quality of life
ICH	International Conference on Harmonisation
ISTH	International Society on Thrombosis and Haemostasis
ITT	Immune tolerance test
kDa	kilodalton

NHP	normal human plasma
PASS	post approval safety study
pdFIX	plasma-derived coagulation factor IX
PEDNET	European pediatric network
PEG	polyethylene glycol
PMDA	Pharmaceuticals and Medical Devices Agency (Japan)
PSS	product-specific standard
PTP	previously treated patient
PUP	previously untreated patient
rFIX	recombinant coagulation factor IX
SAE	serious adverse event
SSC	scientific subcommittee
VAS	visual analog scale
V_{ss}	Volume of distribution at steady-state
WFH	World Federation of Hemophilia

Definition of terms

Exposure day:	Defined as a day where at least one N9-GP dose was administered. This included administration for treatment of bleeds, prophylaxis, surgery, or for pharmacokinetic evaluations.
Patient years of exposure:	Defined as the time in years that individual patients or trial populations were exposed from their first dose to their last dose.
Target Joint:	Defined as a joint having ≥3 bleeds within 6 months.
FIX trough activity	The lowest FIX activity levels in a patient on prophylaxis, i.e., the FIX activity levels in a pre-dose blood sample just prior to next prophylaxis dose.
FIX inhibitors:	Are anti-FIX antibodies that bind to and inhibit or neutralize the functional activity FIX. A patient was per definition positive for FIX inhibitors if two analyses within approximately 14 days were at or above 0.6 BU.

1 Executive Summary

Hemophilia B is an X-linked recessive congenital bleeding disorder, caused by mutations in the coagulation factor IX (FIX) gene, with an incidence of approximately one in every 30,000 male births.[1]

Hemophilia B is a serious, potentially life-threatening disease. The severity of bleeding in hemophilia B is generally correlated with the FIX activity level.[2] Patients with severe hemophilia B with FIX activity below 1% experience bleeds, particularly into joints and muscles, and often without any apparent reason (spontaneous bleeds). Once joint damage has occurred, it will progress over the patient's lifetime even if no further symptomatic bleeds occur in the affected joint.[3] Recurrent bleeds may lead to synovitis, chronic arthropathy, muscular atrophy and deformities, in severe cases leading to significant disability for the patient. Although less frequent, these complications may occur in patients with moderate or mild hemophilia B, who respectively have FIX activity of 2% up to 5% and 5% up to 40%.[2]

There is a need for a therapy for hemophilia B that will restore hemostasis, eliminate or reduce the risk and fear of bleeds, reduce the treatment burden and impact of comorbidities associated with hemophilia (joint disease) and enable normal social and physical function in patients.

There are two main modes of therapy for hemophilia B patients:

- episodic treatment, also referred to as on-demand treatment, and
- preventive treatment, also referred to as prophylaxis.

On demand treatment is the administration of replacement clotting factor given at the time of a bleed to try to stop the bleeding as soon as possible. But recurrent joint bleeding is associated with progressive joint damage, functional impairment and pain, prompting a shift in strategy towards routine factor replacement, known as prophylaxis, to minimize bleeding. Historically, the aim of prophylaxis in hemophilia B has been to keep the FIX activity above 1% to minimize spontaneous bleeds and subsequent joint damage. It is recognized today that treatment to above 1% is not enough, since it does not prevent all bleeding or joint damage. Experts in the field have recommended aiming for a higher factor activity level, with the ideal being non-hemophilia levels (>40%).[4, 5] Maintaining these high levels of FIX is not possible with the currently approved FIX products.

Surgery in patients with hemophilia B is challenging and requires maintaining normal blood clotting capacity or hemostasis for an extended time period beyond the actual surgical procedure, typically achieved by targeting factor activity in the normal range and monitoring for bleeding. Perioperative management with current factor products requires repeated and frequent bolus infusions to sustain sufficient FIX activity levels for hemostasis and wound healing.[2]

Whether for treatment of bleeding, routine prophylaxis, or surgery, current standard factor IX products require multiple intravenous infusions that put a burden on patients and families. Difficulties in accessing veins, in children due to size and in adults due to scarring, complicate treatment. While offering less frequent infusions, current extended half-life products do so without achieving sustained high factor levels.

Clinical rationale for N9-GP

Novo Nordisk has developed N9-GP as an extended half-life FIX product that enables high and sustained FIX levels to be achieved with a less-burdensome once-weekly treatment regimen.

The proposed indication for N9-GP is for use in adults and children with hemophilia B for control and prevention of bleeding episodes, routine prophylaxis and perioperative management.

The N9-GP molecule and its mechanism of action

The Factor IX part of N9-GP (N9) is expressed in a Chinese hamster ovary cell line under serum free conditions without any additional animal- or human-derived materials. A 40 kDa polyethylene glycol (PEG) moiety is conjugated to the N-glycans of the activation peptide of N9 to produce N9-GP. Upon activation, the activation peptide, with the attached PEG moiety, is cleaved off leaving the activated rFIX to form part of the coagulation cascade. Thus, after activation, N9-GP will have the same mode of action and be subjected to the same mechanisms of regulation as endogenous FIXa.

Non-clinical development

The nonclinical program for N9-GP was designed in accordance with relevant international regulatory guidance,[6] and characterized the pharmacodynamics, safety pharmacology, pharmacokinetics, distribution, metabolism, excretion, and toxicology. The nonclinical N9-GP program did not identify any safety concerns.

N9-GP was shown to exert the same mode of action as rFIX, and demonstrated a longer duration of effect in various animal models.

Generally N9-GP was well tolerated in repeat dose toxicity studies. In the rat this included up to 1200 IU/kg every fifth day for 26 weeks and in the cynomolgus monkey up to 1300 IU/kg/week for 4 weeks or 200 IU/kg/week for 13 weeks. The assessments included clinical observations, hematology and biochemistry, as well as histopathological examination of more than 45 organs and tissues, and indicated no specific target organ or systemic toxicity from N9-GP.

PEG safety

PEGylation is a well-established technique commonly used to prolong the circulating half-life of drugs.[7] However, concern has been raised by the FDA about potential PEG accumulation associated with long-term exposure, particularly with respect to the choroid plexus.

In a series of specialized non-clinical studies, N9-GP was not associated with any adverse findings. Immunohistochemistry for PEG and electron microscopy were used to assess the localization and potential cellular effects of PEG. PEG was distributed to the choroid plexus connective tissue and epithelial cells, but not into the brain tissue, documenting that it does not cross the blood-brain barrier. The ultrastructure of the choroid plexus was normal, and PEG was observed in lysosomal vesicles.

The distribution, metabolism and elimination of PEG were examined in studies in mice and rats using N9-GP radiolabelled in the PEG moiety. N9-GP/PEG was distributed rapidly throughout the body, with the highest concentration in well-perfused tissues. Over time, PEG was eliminated from all tissues including the choroid plexus, indicating that PEG will not continue to accumulate but will reach steady-state in plasma and tissues. The distribution data was used to estimate terminal elimination half-lives and time to reach steady-state; i.e., equilibrium between uptake and excretion, and therefore stable concentrations. Steady state was predicted to have been reached in all tissues after a continuous treatment period of 23 weeks in rats. In a 26-week rat toxicity study with chronic N9-GP administration, steady-state was thus reached in all tissues with no observed toxicities.

Applying allometric scaling, terminal tissue elimination half-life and time to steady state were estimated for humans. Time to steady-state in all tissues was predicted to be reached within 2 years with once weekly dosing of N9-GP. As PEG concentrations in human tissues cannot be directly quantified, the rat distribution data was used to build a plasma-tissue PK model to predict PEG concentrations in human plasma and tissues.

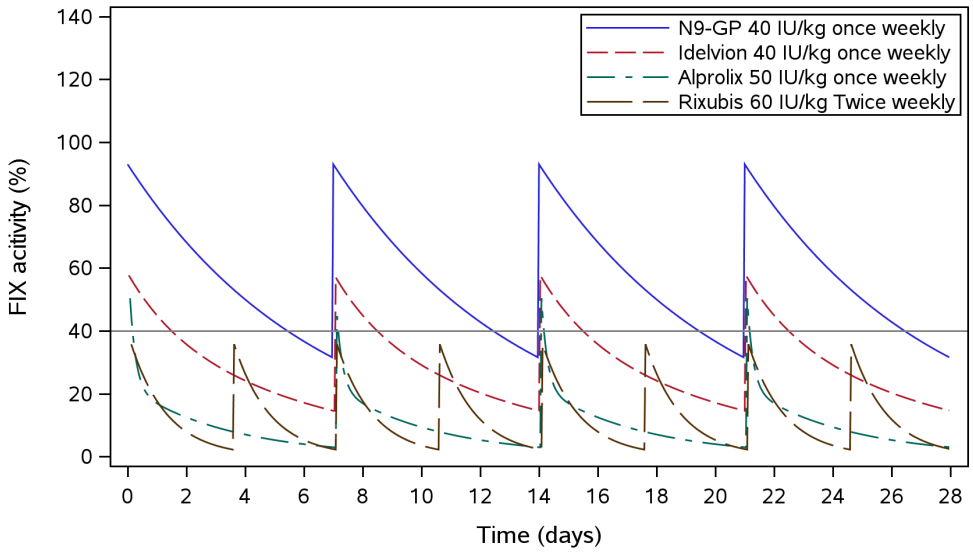
The model predicts that human PEG steady-state concentrations are approximately 7-fold lower than those following chronic N9-GP administration in the 26-week rat toxicity study in which no toxicological findings were observed. This was substantiated by measuring PEG plasma concentrations in clinical samples from children exposed for up to >4 years in the pediatric pivotal trial (Trial 3774); the measured steady-state plasma levels were within the predicted range.

Currently there are 12 approved PEGylated biopharmaceutical products in the US market. Recently published literature reviews on available data conclude that PEG is inert and there are no functional changes or changes with toxicological relevance at clinically relevant doses.[6, 8-13] Thus, more than 20 years of post-authorization experience support the long-term clinical safety of currently marketed PEGylated products.[9, 14] Compared to N9-GP, one of the more relevant products is Cimzia®, which also contains a 40 kDa PEG. Cimzia® is an anti-TNFα compound used chronically for autoimmune conditions. When dosed according to label, Cimzia® would lead to clinical PEG exposures approximately three times higher than those resulting from N9-GP at the proposed clinical dosing of 40 IU/kg once weekly. A meta-analysis of >200 clinical trials concluded that the adverse event profile of Cimzia® does not differ significantly from that of four non-PEGylated anti-TNF agents. To date, there are no reported safety concerns with regards to PEG accumulation arising from post-marketing data from any PEGylated product.

Clinical pharmacology

Two key properties of N9-GP are the significantly prolonged half-life ($t_{½}$) and the increased incremental recovery (change in factor level for every IU administered) of FIX activity compared to all currently approved FIX products. These pharmacokinetics enable patients to maintain consistently high FIX activities with a simplified 40 IU/kg once-weekly dosing schedule. Further, the high levels provide the potential for improved prophylaxis, management of breakthrough bleeds, and perioperative hemostatic control.

With routine once-weekly infusions, 40 IU/kg N9-GP achieved mean steady-state FIX trough levels of 27.3% in adolescents/adults, 19% in children aged 7-12 and 15% in children aged 0-6. Factor IX levels were within the non-hemophilia range (>40%) for 5.4 days (~80%) of the week for adolescents/adults (Figure 1–1) and 2.3 days (~33%) of the week for children. Based upon modeling of published data, the currently approved standard and extended half-life FIX products at their labeled dosing for prophylaxis demonstrate lower recovery and do not sustain as high FIX levels as N9-GP.



The grey line indicates a FIX activity level of 40%.
The predicted pharmacokinetic profiles are based on PK models according to Tiede et al.[15] (N9-GP), Zhang et. al.[16] (IDELVION®), Powell et al.[17] (ALPROLIX®), and RIXUBIS USPI[18].

Figure 1–1 Predicted steady-state FIX activity profiles with once-weekly dosing of N9-GP, IDELVION® and ALPROLIX®, and twice-weekly dosing of RIXUBIS in adults and adolescents

Clinical development program

The N9-GP clinical development program comprises 6 trials in patients with severe or moderate hemophilia B (FIX activity ≤2%): 5 trials (Trials 3639, 3747, 3773, 3775 and 3774) in previously

treated patients, of which Trial 3774 extension phase is ongoing, and 1 trial in previously untreated patients (PUP), which is also ongoing (Table 1–1).

Table 1–1 Overview of N9-GP clinical development program

Trial ID *Status*	Trial design	N9-GP dose (IU/kg) and treatment regimen	Number of patients and patient age
Previously treated patients[a]			
3639 Completed	*First human dose trial* Open-label, dose escalation	25, 50, 100 single-dose	16 patients 21-55 years
3747 Completed	*Pivotal trial* Single-blind, non-controlled, randomized 52 weeks (26 weeks on demand)	Prophylaxis: 10 or 40 once-weekly On demand: 40, 80	74 patients 13-65 years
3773 Completed	*Surgery trial* Open-label, non-controlled	Pre-operative: 80 IU/kg on day of surgery Post-operative: 2 x 40 IU/kg within first 6 days of surgery recommended	13 patients 15-56 years
3775 Completed	*Extension to Trials 3747 and 3773* Open-label, non-controlled	Prophylaxis: 10 or 40 once-weekly 80 once every second week On demand: 40, 80	71 patients 14-66 years
3774 Main phase completed; extension ongoing	*Pediatric trial* Open-label, non-controlled Main phase: 52 weeks	Prophylaxis: 40 once-weekly On demand: 40, 80	25 patients 1-12 years
Previously untreated patients (PUP)[b]			
3895 Ongoing[c]	*PUP trial* Open-label, single-arm, non-controlled	Prophylaxis: 40 once-weekly On demand: 40, 80	50 patients planned <6 years

[a] Patients had received replacement clotting factor prior to entry in N9-GP clinical trials.
[b] Patient had not received replacement clotting factor prior to entry in N9-GP clinical trials.
[c] Ongoing study not included in the biologics license application (BLA)

Patients who were on prophylaxis in the pivotal Trial 3747 were blinded to dose and randomized to 40 IU/kg or 10 IU/kg once-weekly. This allowed for a statistical comparison of the efficacy data between the higher FIX levels obtained with 40 IU/kg once-weekly and the lower FIX levels from 10 IU/kg once-weekly which are comparable to current FIX products. Adolescents/adults transferring to the extension study (Trial 3775) were able to switch between dosing regimens.

Adolescents/adults in the pivotal (Trial 3747) and extension trials (Trial 3775) in need of undergoing major surgery were transferred temporarily to the surgery trial (Trial 3773). After the surgery, they could continue in the extension trial (Trial 3775).

Clinical endpoints and statistical analysis

The primary endpoint in the pivotal trial, the extension trial and pediatric trial was the incidence of FIX neutralizing anti-FIX antibodies (inhibitors) which is considered a serious complication of factor replacement therapy in hemophilia A and B.[19] The primary endpoint was met in these three studies with no inhibitors identified in previously treated patients.

The secondary efficacy endpoints related to treatment of bleeds and prophylaxis were: hemostatic effect of N9-GP when used for treatment of bleeds, evaluated according to a predefined four-point scale (confirmatory endpoint); estimated annualized bleeding rate during prophylaxis (confirmatory endpoint); number of N9-GP infusions used to treat a bleed; consumption of N9-GP; FIX trough activity at steady-state; and patient reported outcomes.

The primary endpoint in the surgery trial was the hemostatic effect during surgery evaluated according to a predefined four-point scale (excellent, good, moderate, poor). The secondary efficacy endpoint related to surgery was consumption of N9-GP.

Efficacy in treatment of bleeds

Overall, there were 591 bleeds with evaluation of hemostatic response across the trials including 341 in the pivotal adult/adolescent trial, 208 in the extension and surgery trials, and 42 in the pediatric trial (Trial 3774 main phase). Across all of the trials and treatment regimens, N9-GP was successful in the treatment of 93.2% of bleeds (92.9% of bleeds in children ages 0-12), which is comparable to or higher than what has been reported for standard and other extended half-life FIX products.[20-23] For adolescents and adults receiving 40 IU/kg once-weekly, 99% of bleeds were treated with one dose of 40 IU/kg. The favorable success rates for treatment of bleeds were obtained with the 40 IU/kg bleed treatment dose without dose adjustment.

Efficacy in Routine Prophylaxis

N9-GP provided low annualized bleeding rates with a simple once-weekly treatment regimen without dose adjustment. Patients treated with 40 IU/kg once-weekly prophylaxis had low ABR in adolescents/adults (estimated mean 2.51, median 1.04) and children (main phase: estimated mean 1.44, median 1.00). The 40 IU/kg once-weekly regimen met the pre-defined statistical criteria for success.

Patients treated with 10 IU/kg once-weekly had an estimated mean ABR of 4.56 (median 2.93), which was higher than for 40 IU/kg (p=0.033); patients treated with 10 IU/kg were 1.9 times more likely to have a bleed (P=0.012) and 2.7 times more likely to have a spontaneous bleed (p=0.014) than patients treated with 40 IU/kg.

An important observed benefit of 40 IU/kg once-weekly prophylaxis was the improvement of target joints, defined at study entry by having ≥3 bleeds in the same joint within 6 months. Patients receiving 40 IU/kg were more likely to have no bleeding in target joints (40 vs 10 IU/kg: 67% vs 8%, p=0.002), and more likely to have target joints without bleeds (71% vs 29%, p=0.031). In

addition, using the ISTH criteria for target joint resolution of ≤2 bleeds into the joint within 12 consecutive months[24], 90% of the 20 target joints in patients on 40 IU/kg were resolved compared with only 58% of the 19 target joints in those treated with 10 IU/kg (p=0.031). For the 6 adolescents/adults who developed 9 target joints while on historical prophylaxis prior to entry in the pivotal trial and were randomized to N9-GP 40 IU/kg once-weekly prophylaxis, 50% of the patients avoided any target joint bleeding during the study and 56% of the target joints did not bleed; in contrast, all of the 7 patients randomized to 10 IU/kg with 11 target joints that developed on prophylaxis prior to the trial experienced target joint bleeding during the trial, and all of the prior target joints continued to have bleeding episodes during the trial. In children, the two patients with baseline target joints, both of whom entered the trial already on routine prophylaxis, had resolution of their target joints with N9-GP 40 IU/kg once-weekly prophylaxis. These results demonstrate that prophylaxis based upon current treatment goals, reflected in the 10 IU/kg once-weekly group, is unable to resolve all target joint bleeding. Furthermore, the advantages of higher FIX levels achieved with the 40 IU/kg dose in preventing bleeding in target joints leads to target joint resolution.

Prophylaxis with 40 IU/kg once-weekly positively impacted health-related quality of life (HRQoL), while 10 IU/kg once-weekly did not. Adult patients (age ≥17) receiving 40 IU/kg once-weekly reported improvements from baseline to end of trial in the overall total score for the HAEM-A-QOL questionnaire, including improvements in the domains 'Feeling', 'Sport' and 'Partnership'. When assessing quality of life using the EQ-5D Visual Analog Scale (VAS), adolescent and adult patients in the 40 IU/kg group had improvement in VAS. There was a significant difference between prophylaxis with 40 IU/kg once-weekly and 10 IU/kg once-weekly in HAEM-A-QoL Overall Score (P=0.049).

Efficacy in perioperative management

Patients with hemophilia B undergoing surgery require normalized hemostasis over an extended period of time past the surgical procedure. The hemostatic effect of N9-GP during surgery was confirmed with an intraoperative success rate of 100% in the 13 major surgeries (Trial 3773), including 9 major orthopedic procedures in patients ages 15-56. In addition, the number of infusions were lower (N9-GP: median 1 pre-op, 4 post-op) than the ranges reported for standard and extended half-life FIX products.[17] Three additional major surgeries were successfully performed during the extension study.

A total of 36 minor surgeries were performed with N9-GP, of which 35 were performed in patients on prophylaxis. In 25 of the 35 minor surgeries (71.4%), patients received a single pre-operative dose of N9-GP 40 IU/kg; in 10 (28.6%) instances the surgery was conducted without an additional pre-operative dose.

N9-GP Clinical Exposure in Previously Treated Patients

A total of 115 unique previously treated patients have been exposed to N9-GP in the completed clinical Trials 3639, 3747, 3773, and 3774 [main phase] (Trial 3775 did not include new patients),

with a total of 8801 exposure days (defined as a day where at least one N9-GP dose was administered). For the completed trials, this corresponds to a total of 170 patient years of exposure through 07 April 2014.

As of 01 November 2016, 20 pediatric patients were still actively participating in the extension phase of Trial 3774 with continuous exposure to N9-GP, and 8 of these 20 children have been treated for more than 4 years (Table 1–2). Furthermore, the trial in previously untreated patients (Trial 3895) is currently ongoing where duration of exposure in a patient has exceeded 2 years. For the completed and ongoing trials, this corresponds to a total of 226 patient years of exposure through 01 November 2016.

Table 1–2 Number of patients exposed to N9-GP by age and duration of treatment – from completed and ongoing trials in previously treated patients

Duration of exposure by age	0-6 years (N=12)	7-12 years (N=13)	13-17 years (N=18)	18-70 years (N=72)	Total (n=115)
0 - <12 months	1	2	1	20	**24**
12 - <24 months	0	0	4	25	**29**
24 - <36 months	1	1	13	27	**42**
36 - <48 months	8	4	0	0	**12**
>=48 months	2	6	0	0	**8**

Exposure for patients in Trial 3774 is calculated up until latest dose before 01 November 2016.
Additional safety data from the ongoing extension phase of Trial 3774 (i.e., from cut-off date 01 April 2016 in the 120-day Safety Update to cut-off 01 November 2016 for this document) has not been evaluated by the FDA.

Clinical safety

Specific pharmacological risks for FIX replacement products include FIX inhibitors, allergic-type hypersensitivity reactions, and thrombotic events, which were evaluated in all trials.

No clinical safety concerns were identified with use of N9-GP and the nature and frequency of the reported adverse events did not reveal any unexpected safety signals.

As of 01 November 2016, none of the previously treated adult, adolescent or pediatric patients in completed or ongoing trials have developed FIX inhibitors.

A previously untreated pediatric patient developed a FIX inhibitor, not unexpected given the incidence of inhibitors in previously untreated patients with severe hemophilia B (5-10%).[19] Allergic-type hypersensitivity reactions, including anaphylaxis, were reported in 4 patients (5 events), which is consistent with the occurrence with other FIX products.[19, 25]

No thromboembolic events were reported.

Overall, adverse event rates were similar to what could be expected from studies in a population of patients with severe and moderately-severe hemophilia B and consistent with the safety profile of other FIX products.

Assessments of hematology, biochemistry, coagulation-related parameters and urinalysis did not indicate any clinically relevant changes associated with exposure to N9-GP. No safety concerns were identified from vital signs, physical examinations or ECGs.

Benefit-risk evaluation

Based on published data, N9-GP provides and maintains higher FIX levels than those delivered by the current standard and extended half-life products with a convenient once-weekly dose. N9-GP 40 IU/kg once-weekly achieved non-hemophilia factor IX levels for approximately 80% of the week for adolescents/adults; on average, children were consistently above 15% factor IX activity for the entire study period, and older children were above 19%.

The high factor IX levels obtained with N9-GP 40 IU/kg once-weekly prophylaxis results in: reduced annualized bleeding rates in adolescents and adults with median ABR 1.04 and children with median ABR 1.00; reduced annualized spontaneous bleeding rates (AsBR) in adolescents and adults with median AsBR 0.0 and in children median AsBR 0.0; resolution of 90% of target joints in adolescents and adults and 100% in children, and enhanced quality of life.

Furthermore, N9-GP provided successful bleed control in 97% of adolescent and adult patients and in 93% of children who were receiving prophylaxis with once-weekly with 40 IU/kg. N9-GP provided effective perioperative management in all 16 major surgeries, and in the minor surgeries where efficacy was evaluated.

Through the clinical development program, N9-GP has demonstrated a safety profile similar to that of currently approved Factor IX products, with no inhibitors in previously treated patients, no thromboembolic events, and an expected rate of allergic reactions.

Non-clinical and clinical data support the safety of N9-GP. Non-clinical data have shown that PEG is eliminated and indicate that PEG steady-state concentrations will be reached in patients within 2 years in plasma and all tissues. This was substantiated by PEG plasma concentration data in clinical samples from pediatric patients documenting that steady-state plasma levels were achieved within the predicted range.

In the nonclinical studies, no adverse histological changes were observed in tissues, including the choroid plexus. Based on the now >4 years of active and ongoing treatment in children, no unexpected safety concerns have been identified and N9-GP has presented a safety profile similar to that of other Factor IX products. While uncertainty always exists around long term safety of new

drugs at the time of registration, the long term PEG safety related to N9-GP is supported by the significant post-marketing experience with PEGylated proteins, some of which have been in clinical use for more than 20 years. The nonclinical data and clinical experience with N9-GP have not to date shown any adverse consequences. However, in consideration of the limitations of long term clinical safety and exposure data prior to approval, the risk management plan includes measures to enhance the detection of potential adverse reactions.

N9-GP provides clinically meaningful benefits for control and prevention of bleeds, perioperative management, and routine prophylaxis which outweigh the uncertainty around the theoretical risk pertaining to chronic exposure to the PEG moiety.

2 Background

Summary

- Severity of bleeding in hemophilia B is generally correlated with the FIX activity. Patients with severe hemophilia B have factor IX activity of less than 1% and experience bleeds, particularly into joints and muscles; these bleeds often occur without any apparent reason or trauma, and are known as spontaneous bleeds.
- Based upon recognition of the risk and consequences of bleeding, management of severe and moderately-severe hemophilia have evolved from treating bleeding episodes, called on demand therapy, to routine factor replacement infusions to prevent bleeding, called prophylaxis.
- Recent studies have highlighted that routine infusions to keep factor activity above 1%, outside of the range of severe hemophilia, is not sufficient to avoid all bleeding, or to prevent radiographic or clinical progression of joint disease.
- Epidemiologic studies of mild and moderate hemophilia suggest that bleeding risk exists with baseline factor activity of up to 15-20%.
- Based upon epidemiologic and prophylaxis studies, experts have recommended targeting higher factor levels with prophylaxis, including at least minimum of 15-20%, and ideally in the non-hemophilia range of above 40%.
- At labeled doses, available standard and extended half-life factor IX products are not able to achieve high factor activity levels in the non-hemophilia range for a substantial portion of the dosing interval.
- There remains an unmet patient need for a treatment that can provide substantially higher factor IX levels within the context of a less frequent dosing interval of at least a week.
- N9-GP is a recombinant FIX molecule with a 40 kDa polyethylene glycol (PEG) moiety attached to the activation peptide providing 5 times longer half-life and higher recovery of factor IX activity.

2.1 Disease background and unmet need

Hemophilia B is an X-linked recessive congenital bleeding disorder, caused by mutations in the coagulation factor IX (FIX) gene, with an incidence of approximately one in every 30,000 male births.[1] The gene-defects are transferred from a heterozygotic mother (carrier) or ascribable to new, spontaneous mutations. More than 50% of all patients with hemophilia B have no known family history of the disease, and these are called sporadic or isolated cases.[26-30]

Hemophilia B is a serious, potentially life-threatening disease. The severity of bleeding in hemophilia B is generally correlated with the FIX activity level.[2] Patients with severe hemophilia B have FIX activity below 1% and often experience spontaneous bleeds, which occur particularly into joints and muscles; those occurring without any apparent reason are known as spontaneous bleeds. Once joint damage has occurred, it will progress over the patient's lifetime even if no further symptomatic bleeds occur in the affected joint.[3] Although less frequent, these complications

may occur in patients with moderate or mild hemophilia B with FIX activity of 1 to 5% and 5 to 40%, respectively.[2]

Recurrent bleeds may lead to synovitis, with synovial inflammation setting up a cycle of recurrent bleeding into joints. Joints that have ≥3 bleeds within 6 months are known as target joints.[2] Damage to the joint progresses from the soft tissues changes of acute synovitis to chronic arthropathy as bone and cartilage become involved. As function deteriorates, muscles atrophy and fixed deformities occur, in severe cases leading to significant disability for the patient.

There is a need for a therapy for hemophilia B that will restore hemostasis, eliminate or reduce the risk and fear of bleeds, reduce the treatment burden and impact of comorbidities associated with hemophilia (joint disease) and enable normal social and physical function in patients.

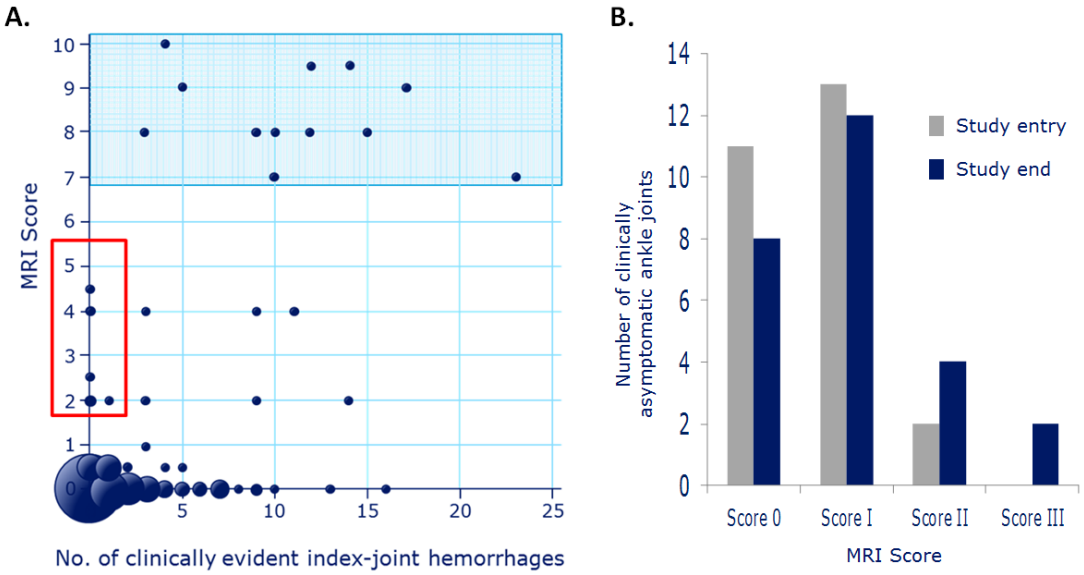
There are two main modes of therapy for hemophilia B patients, episodic treatment (on demand) and preventative treatment (prophylaxis). On demand treatment is the administration of replacement clotting factor given at the time of a bleed to try to stop the bleeding as soon as possible. But recurrent joint bleeding is associated with progressive joint damage, functional impairment and pain, prompting a shift in strategy towards routine factor replacement, known as prophylaxis, to minimize bleeding.

Historically, the aim of prophylaxis in hemophilia B has been to keep the FIX activity above 1%, essentially out of the range of severe hemophilia, to minimize spontaneous bleeds and subsequent joint damage. The initial movement towards prophylaxis was focused on children, where institution of prophylaxis prior to joint bleeding (primary prophylaxis) was advocated as a way to prevent joint bleeds and minimize potential for joint damage.

It is recognized today that treatment to a pre-dose minimum factor activity or trough level of above 1% is not enough, since it does not prevent all bleeding or joint damage.

- Data from the landmark Joint Outcome Study over the initial 6 year period showed that while patients on primary prophylaxis had less bleeds and joint damage than those treated on demand, there were patients on prophylaxis even without clinical bleeding that developed soft tissue changes on MRI scans of the knees/ankles/elbows (Figure 2–1A) and evidence of iron deposition, promoting the notion that there must be microbleeds occurring that patients didn't recognize.[31] Even those treated successfully with routine prophylaxis to minimize bleeding continued to have MRI changes through age 14.[32]
- A single-center study of asymptomatic ankles in 26 boys with hemophilia A and B on standard prophylaxis for a mean of 8 years follow-up demonstrated MRI changes in ankles that had not overtly bled (Figure 2–1B).[33]
- A retrospective epidemiologic study of up to 6 years and a prospective epidemiologic study of up to 25 years showed joint damage even with successful prophylaxis to above 1% target. The retrospective study showed that while some patients with factor levels below 1% did not bleed, others with trough levels during treatment of above 3% continued to have bleeds.[34]

The prospective study showed a combination of both clinical and radiographic evidence of joint damage (Table 2–1).[35]



Red box highlights patients who had no clinically evident bleeding in any of 6 index joints (knees, ankles, elbow) but had abnormal MRI scores.

Figure 2–1 MRI joint changes in children on prophylaxis (A) Joint Outcome Study of 6 index joints through age 6 (B) Single center study of asymptomatic ankles up to 8 years follow-up

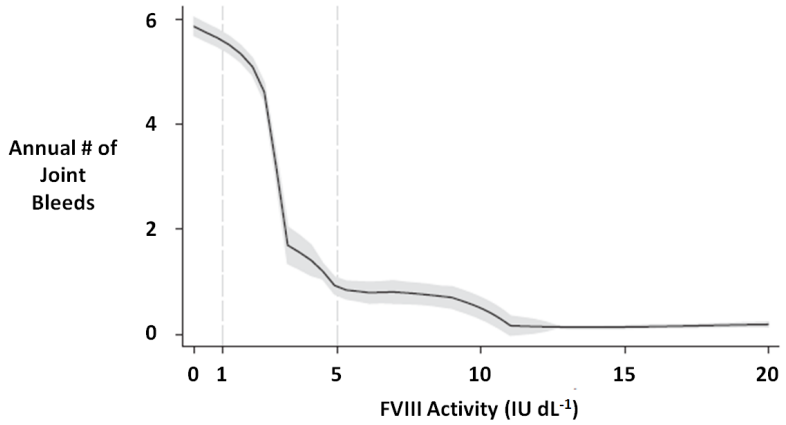
Table 2–1 Joint bleeding, clinical and radiographic joint abnormalities in children on prophylaxis up to 25 years

	Present Age in Years				
	3-6 N=6	**7-12** N=9	**13-17** N=20	**18-23** N=10	**24-32** N=15
Age prophylaxis started	1.1 (1-1.5)	1.2 (0.5-2)	2.6 (1-4.5)	4.9 (3-7)	7.0 (3-13)
Annual joint bleeds	0.1 (0-6)	0.1 (0-0.4)	3 (0.1-16.6)	5.6 (0.5-14)	5.0 (1.6-16)
Orthopedic joint score	0	0	1.2 (0-7)	2.9 (0-7)	6.6 (0-15)
Radiological joint score	0	0	4.8 (0-22)	14.2 (0-22)	20.6 (0-41)

Modified from Nilsson et al., 1992[35]

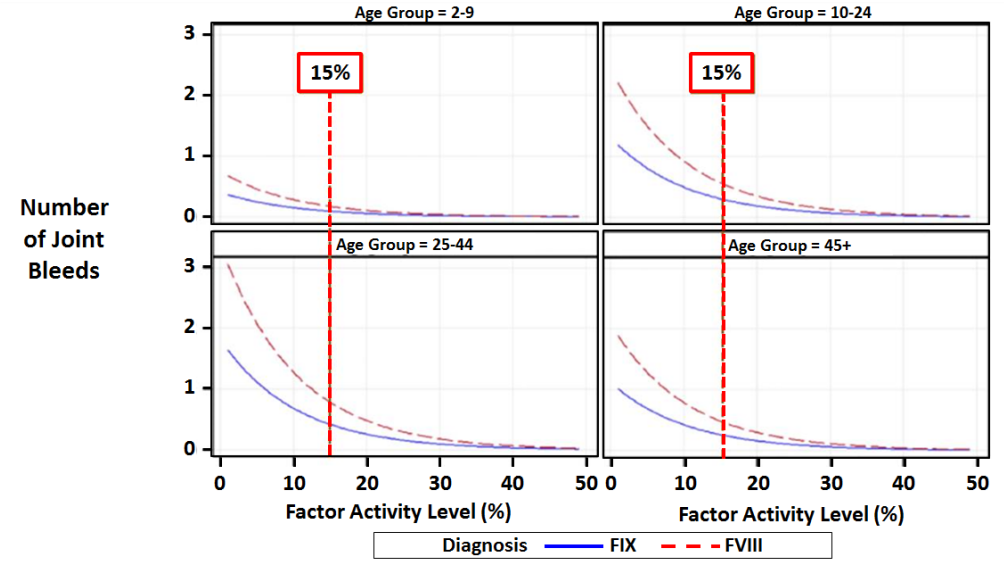
While there have been no prospective studies to resolve the issue of what target of prophylaxis would better prevent joint damage, epidemiologic studies in patients with mild to moderate hemophilia treated on demand provide insights into the relationship of baseline factor activity and risk of bleeding.

- In a study of 433 patients with hemophilia A in the Netherlands treated on demand with mild (73%) or moderate (27%) hemophilia, joint bleeding was seen to correlate with factor VIII activity (Figure 2–2). Risk of bleeding declined 18% for every 1% increase in FVIII, and those with levels 15% or above would be expected to have no joint bleeds.[36]
- In a public health surveillance study of 3315 and 1456 patients with hemophilia A and B respectively aged 2-91 in Hemophilia Treatment Centers in the US for annual visits for up to 12 years, regression modeling suggest joint bleeds would be expected to occur at increasing factor VIII or IX levels based upon increasing age (Figure 2–3). Modeling predicted 0.6 bleeds per year for patients with hemophilia B with a baseline FIX level of 15%.[37] In the same US study, ongoing surveillance of those with mild and moderate hemophilia, the majority treated with an on demand regimen, demonstrates presence of target joints reflecting frequent bleeding and patients requiring invasive joint procedures.[38]



Modified from den Uijl et al., 2011[36]

Figure 2–2 Number of joint bleeds per year based upon baseline FVIII activity (Netherlands)



Modified from Soucie et al., 2015[37]

Figure 2–3 Number of joint bleeds per year based upon baseline FVIII or FIX level (US)

Table 2–2 Presence of target joints and invasive joint surgeries in mild to moderate hemophilia (US)

	Hemophilia A		Hemophilia B	
	Mild N=4108	Moderate N=2721	Mild N=1196	Moderate N=1491
At least one target joint	3.0%	14.6%	2.8%	6.4%
At least one joint procedure	54	73	19	24

Further, there is an acknowledgement that active patients with hemophilia can sustain bleeding even with minor traumas and thus require higher factor activity levels to be able to remain active. The specific correlation with bleeding risk and activity has only been investigated in limited studies. One study of 104 boys, who were participating in contact sports and evaluated for bleeding up to 8 hours after the activity, showed the incidence of bleeds was 2% lower for every 1% increase in factor level at the time of the activity.[39] For activities where collisions might occur, the odds of bleeding were increased 2.7 fold and would be normalized by factor activity exceeding ~55%. For sports where collisions are inevitable, the risk was 3.7 fold higher and normalized only by activity exceeding ~ 70%.

Based upon these results, experts in the field have recommended aiming for a higher factor activity level, with the ideal being non-hemophilia levels (>40%).[4, 5] Maintaining these high levels of FIX activity are not possible with the currently approved FIX products; thus discussion has focused on

various targets, including maintaining a range of 15-20% based upon the epidemiologic studies discussed above.

Surgery in patients with hemophilia B is challenging due to high risk of excessive bleeding, and requires normal hemostasis for an extended time period. Current perioperative treatment requires repeated bolus infusions of replacement clotting factor to sustain sufficient FIX activity levels for hemostasis and wound healing.[2] Due to the pharmacokinetic characteristics of currently available standard and extended half-life rFIX products, frequent administration is required; one extended-half life FIX required 5-27 infusions in the first 14 days post-op, similar to standard FIX concentrate that required 7-31 infusions.[40]

Development of neutralizing anti-FIX antibodies (inhibitors) is a serious complication of FIX replacement therapy.[19, 25, 41, 42] Among patients with severe hemophilia B, 5-10% will develop inhibitors following exposure to Factor IX.[19] Inhibitors in hemophilia B develop after relatively few treatments (exposures) to a FIX product since the patient with hemophilia does not recognize the FIX protein as "self". In patients who develop inhibitors to FIX, the condition will manifest itself as an insufficient clinical response to FIX replacement therapy. The occurrence of FIX inhibitors also shows some correlation to allergic reactions, and patients with FIX inhibitors are at an increased risk of anaphylaxis with subsequent exposure to FIX (~50% of the patients) as well as development of nephrotic syndrome if re-exposed to Factor IX during immune tolerance therapy (ITT).[43]

Whether for treatment of bleeding, routine prophylaxis, or surgery, current standard factor IX products require multiple intravenous infusions that put a burden on patients and families. Difficulties in accessing veins, in children due to size and in adults due to scarring, complicate treatment. While offering less frequent infusions, current extended half-life products do so without achieving sustained high factor levels.

N9-GP was developed in order to offer a more effective and less burdensome treatment by prolonging the half-life and improving the overall pharmacokinetic profile of the molecule as compared to other FIX products. With the prolonged half-life and sustained high FIX activity, N9-GP has the potential to improve prophylaxis and simplify management of bleeds as well as simplifying perioperative hemostatic control in patients with hemophilia B.

2.2 Key properties and mechanism of action

The N9-GP drug product is a lyophilised powder provided in single-use vials to be reconstituted with 10 mM histidine solution for intravenous infusion.

N9-GP is a recombinant FIX molecule expressed in a genetically engineered Chinese hamster ovary cell line and the production process is without any human- or additional animal-derived materials. A 40 kDa polyethylene glycol (PEG) moiety is attached to the FIX activation peptide by site-directed glycoPEGylation. This PEGylation, which is a well-known method of modifying proteins,

increases the half-life of the protein by reducing elimination processes such as renal excretion, receptor mediated uptake, and proteolytic degradation.

Upon activation of N9-GP during the coagulation process, the activation peptide of the molecule is cleaved off leaving the activated FIX (FIXa), which is identical to native FIXa. Together with activated factor VIII (FVIIIa), FIXa forms the tenase complex on the surface of activated platelets resulting in the activation and conversion of factor X (FX) into FXa. This then leads to the conversion of prothrombin into thrombin which converts fibrinogen into fibrin and enables the formation of a hemostatic plug.

The mechanism of action for N9-GP is based on replacement of the deficient, lowered, or absent FIX in patients with hemophilia B. By injecting FIX intravenously, the impaired coagulation process is re-established, and enables the formation of a stable hemostatic plug in response to vascular damage.

The functionality of the activated rFIX derived from N9-GP (i.e., when the activation peptide containing the glycoPEGylation has been cleaved off) was assessed in *in vitro* studies using plasma and whole blood, and was demonstrated to exhibit primary pharmacodynamic properties comparable to non-PEGylated rFIX. After activation there is no biological role of the activation peptide which is proteolytically degraded, leaving PEG in the circulation until excreted via urine and feces.

2.3 Regulatory guidance and advice

The clinical development of N9-GP was based on relevant guidelines, including the European Medicines Agency (EMA) guideline on the clinical investigation of recombinant and plasma-derived factor IX products[44] and the guideline from the International Society on Thrombosis and Haemostasis (ISTH).[45] In addition, the trials were performed in accordance with the Declaration of Helsinki and ICH Good Clinical Practice.

Development of N9-GP has been guided by interactions with FDA (US), as well as EMA (Europe), PMDA (Japan) and Health Canada, all of whom have provided scientific advice to the phase 3 clinical trial program.

3 Nonclinical data

Summary

- N9-GP was shown to exert a similar mode of action as rFIX, but with 2-6 fold increased half-life and longer duration of effect.
- At very high doses (3750 IU/kg/week), transient tremors were seen in cynomolgus monkeys. The no observed adverse effect level was 1300 IU/kg/week. The clinical dose is once weekly 40 IU/kg for prophylaxis.
- Toxicology studies up to 26 weeks duration in rats with 1200 IU/kg every fifth day did not identify any adverse findings or histological changes.

3.1 Overview of nonclinical program

The nonclinical program for N9-GP includes primary pharmacodynamics, safety pharmacology, pharmacokinetics, distribution, metabolism, excretion, toxicology and other supportive studies (Table 3–1). All pivotal safety studies were conducted according to Good Laboratory Practice (GLP) and relevant international regulatory guidance.[6, 46, 47]

Table 3–1 Overview of N9-GP nonclinical program

Discipline	Type of study	N9-GP doses (IU/kg)	Corresponding PEG doses (µg/kg/week)	Species
Pharmacodynamics				
In vitro efficacy	Functional *in vitro* characterization	NA	NA	NA
In vivo efficacy	Acute and prolonged effect in various bleeding models	1.5-250	9-1438	Factor IX knockout (F9-KO) mouse
Safety pharmacology	Respiratory, cardiovascular, renal and central nervous system in a 4 week repeat-dose toxicity study	350, 1300 and 3750 (once-weekly)	2013, 7475, 21563	Cynomolgus monkey
Pharmacokinetics				
Single-dose PK	Single-dose	Range: 30-2000	Range: 173-11500	F9-KO mouse, rat, dog, minipig and cynomolgus monkey
Multiple dose PK	4-26 weeks toxicology studies)	Range: 40-3750	Range: 230-21563	Rowett nude rat, cynomolgus monkey
Distribution Single-dose	Quantitative whole body autoradiography – followed for 12 weeks	Range: 127-238	Range: 600 -1200	F9-KO mouse, rat[48]
Metabolism Single-dose	HPLC and gel electrophoresis of plasma, urine and feces followed for up to 12 weeks	Range: 120-238	Range: 600-1200	F9-KO mouse, rat[48]
Excretion Single-dose	Collection of urine and feces for 12 weeks	Range: 25-238	Range: 100-1200	F9-KO mouse, rat[48]
Toxicology (highest level with no toxicity in shown in bold)				
Single-dose toxicity		200, 1000, **2000**	1150, 5750, 11500	Rat
Repeat-dose toxicity	6 weeks	0, 40,**1200** (twice weekly)	0, 460, 13800	Rowett nude rat[49]
	26 weeks	0, 40, 150, 600,**1200** (every fifth day)	0, 322, 1207, 4830, 9660	Rowett nude rat[49]
	4 weeks	0, 350, **1300**, 3750 (once-weekly)	0, 2013, 7475, 21563	Cynomolgus monkey
	13 weeks	200 (once-weekly)	1150	Cynomolgus monkey
Local tolerance	Single and repeat-dose local tolerance	40-3750	230 – 21563	Rabbit, Rowett nude rat Cynomolgus monkey
Other toxicity	Comparative immunogenicity to BeneFIX	25 and 200	144-1150	Rat

3.2 Pharmacodynamics

In vitro assessments of the general mechanism of action showed that N9-GP is activated by its normal physiological activators (i.e., coagulation factor XIa and tissue-factor-coagulation factor VIIa) as in the normal coagulation cascade. Once N9-GP has been activated and the activation peptide is cleaved off, the pharmacological properties were indistinguishable from those of rFIXa. After activation there is no biological role of the activation peptide, which is proteolytically degraded. In *in vivo* studies, N9-GP was shown to have 2-6 fold increased half-life and exert similar pharmacodynamic properties to those of rFIX, including hemostatic effect, but with a substantially longer duration.

3.3 Safety pharmacology data

Safety pharmacology endpoints (respiratory, cardiovascular, renal and central nervous system) were evaluated in the 4 week repeat-dose toxicity study in cynomolgus monkeys. No safety signals were identified with doses up to 1,300 IU/kg/week. The safety pharmacology endpoints were assessed pre-treatment and for 1-3 hours after each dose. The only treatment related finding was mild and transient body tremors in the high dose group (3750 IU/kg). Tremors were observed at a single or two occasions during neurological assessment or clinical observation and not beyond the third dose. As tremors were seen only at early time-points, it was considered unlikely that these were related to accumulation of PEG. A cause for the tremors was not identified.

There were no effects on any of the safety pharmacology parameters up to and including 1300 IU/kg, which was therefore considered the NOAEL for this evaluation. Tremors were not seen during clinical development.

3.4 Pharmacokinetics

N9-GP showed a monophasic elimination pattern in a number of nonclinical species. Systemic exposure increased proportional to dose and clearance, and was scalable and predictable for clearance in humans. Like other PEGylated proteins, the protein part of N9-GP showed a profile compatible with degradation via proteolysis by lysosomal enzymes.

Distribution, metabolism and excretion of the N9-GP PEG moiety are described in more details in Section 4.3.

3.5 Toxicology

The toxicity of N9-GP was assessed in the rat with single-dose administration up to 2,000 IU/kg (highest dose tested) and repeat-dose administration up to 1,200 IU/kg every fifth day for up to 26 weeks (highest dose and longest duration tested). Potential N9-GP toxicity was also studied in the cynomolgus monkey with repeat-dose administration up to 3,750 IU/kg/week in a 4-week study. Clinical observations, hematology and biochemistry as well as histopathological examination of >45 organs and tissues from the rat and cynomolgus monkey repeat-dose toxicity studies indicated no specific target organ or systemic toxicity from N9-GP up to 1,200 IU/kg/5d in rat and up to

1,300 IU/kg/week in cynomolgus monkey. For comparison to the clinical dose please refer to Table 3–2.

Similar to what has been observed for other human coagulation factors, dosing beyond approximately 4 weeks caused development of cross-reacting neutralizing antibodies in the monkey. This resulted in acquired hemophilia and decreased exposure, thus precluding the use of monkey for chronic toxicity studies. Therefore, the Rowett nude rat was introduced to circumvent the immune system in order to be able to assess chronic toxicity of N9-GP. The Rowett nude rat lacks the thymus and is therefore unlikely to produce anti-drug-antibodies.

Table 3–2 Fold from nonclinical dose not causing adverse findings to a once weekly clinical dose of 40 IU/kg N9-GP (~230 µg/kg/week PEG in the prophylactic regimen)

Duration of nonclinical study	Nonclinical N9-GP dose not causing adverse findings (IU/kg)	Corresponding nonclinical PEG dose not causing adverse findings (µg/kg/week)	x-fold higher compared to the 40 IU/kg once weekly clinical dose based on mg/kg PEG	x-fold higher compared to the 40 IU/kg once weekly clinical dose based on HED for PEG (conversion to body surface area)[b]	Species
6 weeks	1200 IU/kg/twice weekly[a]	13800	60	10	Rowett nude rat
26 weeks	1200 IU/kg/5d[a]	9660	42	7	Rowett nude rat
4 weeks	1300 IU/kg/wk	7475	32	10	Cynomolgus monkey
13 weeks	200 IU/kg/wk[a]	1150	5	2	Cynomolgus monkey

[a]Highest dose tested

[b]Conversion based on body surface area, human equivalent dose (HED), calculated by multiplying dose with 0.32 (monkey) and 0.16 (rat).[50]

In conclusion, N9-GP was well tolerated in the monkey up to 1300 IU/kg/week and in the rat up to 1200 IU/kg every fifth day. No treatment related toxicity findings were observed at any dose or duration of dosing in the non-clinical studies.

4 PEG Safety

Summary

- No adverse findings in any tissue were associated with PEG from N9-GP
- PEG was not observed in the brain; i.e., PEG did not cross the blood brain barrier
- The ultrastructure of the choroid plexus appeared normal; lysosomes with PEG micro-vesicles were observed but not considered adverse.
- PEG from N9-GP was shown to be distributed to and eliminated from all tissues, including the choroid plexus, and excreted in urine and feces.
- PEG from N9-GP will reach steady-state in plasma and tissues and will not continue to accumulate.
- PEG plasma levels measured in pediatric patients treated for up to 4.5 years with N9-GP confirmed plasma-tissue model predictions that steady state was achieved.
- The predicted steady-state concentrations in human tissues were approximately 7-fold lower than those achieved in a 26-week chronic toxicity study in rats where no adverse findings were observed.
- There were no unexpected safety findings, including renal, hepatic, or neurologic, for N9-GP in long term clinical studies with greater than 4 years exposure.
- Cellular vacuolation has been observed with other PEGylated products. No PEG related vacuoles were seen in any of the N9-GP nonclinical studies.
- Based on data from a range of marketed PEGylated products, PEG is inert and there are no functional changes or changes with toxicological relevance at clinically relevant doses.
- More than 20 years of post-authorization experience support the long-term clinical safety of currently marketed PEGylated products

4.1 Background

FDA has questioned the potential impact of 40 kDa PEG accumulation following long-term dosing of N9-GP, and in particular the significance of PEG observed in the choroid plexus in monkeys and rats from the nonclinical N9-GP program. The choroid plexus is a multi-lobed vascular structure in the ventricles of the brain and produces cerebrospinal fluid. A series of specialized investigations were conducted assessing the detailed distribution, elimination and potential effects of PEG following N9-GP dosing. These data were compared to the extensive literature on PEG.

4.2 Histological assessments

As described in Section 3.5, N9-GP was not associated with PEG related adverse effects in nonclinical toxicity studies in rat and monkey with up to 26 weeks and 13 weeks dosing, respectively.

Histopathological examinations of more than 45 tissues did not identify any adverse findings and all tissues were normal. There was a particular focus on potential cellular vacuolation as this has been observed with other approved PEGylated biopharmaceuticals (Section 4.7). No PEG-related vacuolation was seen with N9-GP administration.

In addition to the standard toxicological and histopathological investigations, and based on literature and regulatory guidance,[6, 51] specific sensitive techniques for detection of PEG (immunohistochemical staining and electron microscopy) were performed on brain tissue and choroid plexus from the N9-GP repeat-dose toxicity studies ().

Table 4–1 Repeat-dose toxicity studies – results from histopathology and special investigations

Study	N9-GP dose (~ IU/kg)	Corresponding PEG dose (µg/kg/week)	Histopathology (light microscopy of >45 tissues)	PEG immunohistochemistry (IHC) and Electron Microscopy (EM) brain tissue and choroid plexus
26 weeks in rat	*Main 26 weeks*: 0, 40, 150, 600, 1200 *Recovery 26 weeks* 0, 1200 *Dose frequency:* Every fifth day	0, 322, 1207, 4830, 9660	No histopathological changes	IHC: PEG detected in choroid plexus connective tissue and epithelial cells at all dose levels (40-1200 IU/kg N9-GP) PEG not detected in brain tissue EM: PEG presence in micro-vesicles in lysosomes of choroid plexus epithelial cells. No ultra-structural changes observed (1200 IU/kg N9-GP)
4 weeks in monkey	*Main 4 weeks:* 0, 350, 1300, 3750 *Recovery 5 weeks*: 350, 1300 *Recovery 1 week*: 3750 *Dose frequency:* Once weekly	0, 2013, 7475, 21563	No histopathological changes – except hemorrhages as sign of acquired hemophilia due to cross reacting neutralizing antibodies in high dose.	IHC: PEG not detected in animals dosed 350 IU/kg/week N9-GP. PEG detected in choroid plexus connective tissue and epithelial cells (1300, 3750 IU/kg N9-GP). PEG was not detected in brain tissue.
13 weeks in monkey	*13 weeks treatment + 5 weeks recovery:* 200 *Dose frequency:* Once weekly	1150	No histopathological changes	IHC: PEG was not detected in brain tissue or choroid plexus

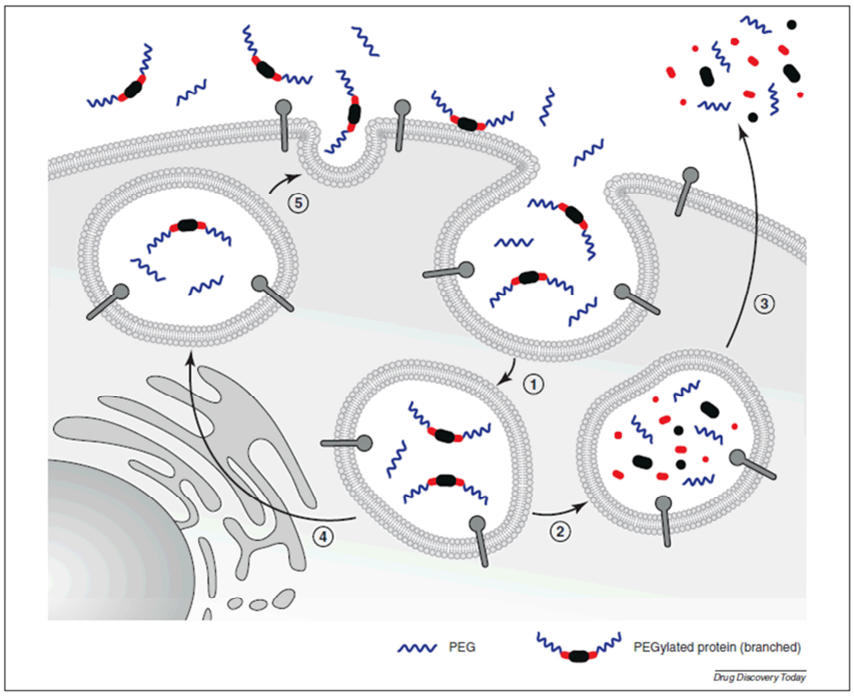
Using PEG specific immunohistochemical staining, PEG was shown to be distributed to connective tissue and epithelial cells of the choroid plexus in both rat and monkey. PEG was not detected in

any other brain structures. The ultrastructure of the choroid plexus appeared normal. Lysosomes with PEG micro-vesicles were observed, but there were no signs of adverse cytological effects in choroid plexus epithelial cells (i.e., the structures of microvilli, tight junctions, mitochondria, rough endoplasmic reticulum, and the Golgi complex were normal). Therefore, the presence of PEG in lysosomes in the choroid plexus was not considered adverse.[49]

4.3 Distribution, excretion and metabolism of PEG from N9-GP

While immunohistochemistry enables visualization of PEG and electron microscopy allows for detection of potential changes in cellular structures, these techniques are not quantitative. Therefore the fate of the 40 kDa PEG moiety of N9-GP was investigated via distribution, metabolism and excretion studies in mice and rats, conducted with N9-GP radiolabelled in the PEG moiety. These studies allow quantification of PEG concentration in tissues, urine and feces over time.

Metabolism and excretion studies showed that the protein part of N9-GP was degraded over time as expected, leaving 40 kDa PEG in circulation until excreted in urine (~42-56%) and feces (~28-50%).[48] Overall, the kinetic properties of the PEG moiety of N9-GP were similar to those reported in the extensive review of pharmacokinetics, distribution and metabolism of PEG by Baumann et al. in 2014.[7] Thus, PEGylated proteins undergo uptake into cells by pinocytosis, degradation of the protein part in the lysosomes/endosomes, and excretion of the PEG part back to plasma via exocytosis and cellular turnover (Figure 4–1).[7]



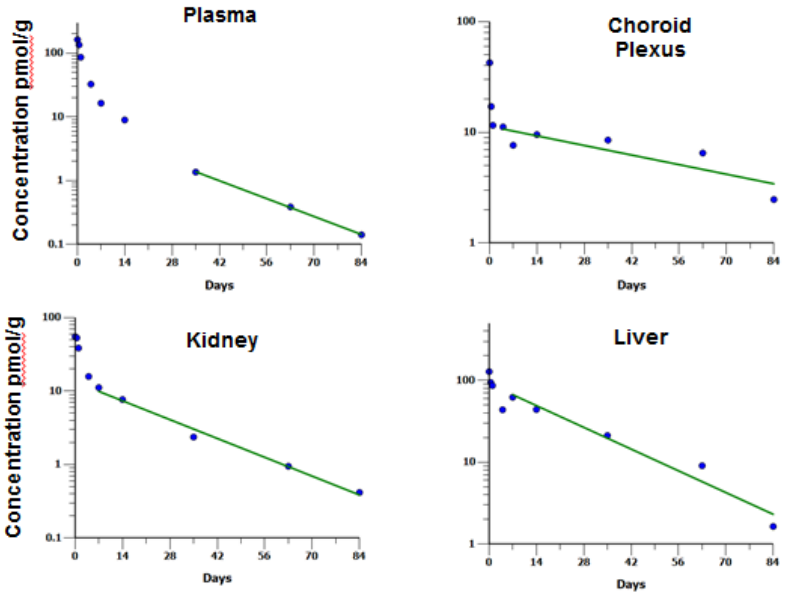
A PEGylated protein or PEG can be taken up by the cell via pinocytosis. The PEGylated protein or PEG is internalized by the cell (1) and can be transported to the endosomes/lysosomes (2) where it can be metabolized in the acidic environment. Metabolic products including PEG can be released from the cell by exocytosis (3). The PEGylated protein or PEG can also be released from the cell without having undergone any metabolism (4 and 5). Image is copied from Baumann et al. 2014.[7]

Figure 4–1 Cellular processes involved in uptake, metabolism and excretion of PEGylated proteins

In distribution studies, N9-GP was distributed rapidly throughout the body, with the highest concentration in well-perfused tissues including liver, kidney and choroid plexus. Over time, PEG from N9-GP was eliminated from all organs including the choroid plexus. There was no indication that N9-GP or PEG crosses the blood brain barrier or enters the brain tissue. This is consistent with the observed immunohistochemical PEG staining from the repeat-dose toxicity studies in rat and cynomolgus monkey where PEG was detected within the blood vessels of the brain but not in the brain parenchyma.

4.4 Terminal elimination half-life and time to steady-state of the PEG moiety of N9-GP

The data from the rat distribution study were used to estimate the terminal elimination half-lives of PEG in tissues by non-compartmental analysis. This was done by fitting a regression line to the terminal elimination phase of the observed data (Figure 4–2).



PEG concentration versus time plots showing actual data (dots) and regression lines (green line) used to determine terminal elimination half-life of PEG for plasma, kidney, choroid plexus and liver. From the plasma and tissue data, non-compartmental analysis of N9-GP indicates that the majority of exposure (AUC) was captured (99, 98, 97 and 72% for plasma, kidney, liver and choroid plexus, respectively) and hence the regression line represents the true terminal phases. Quantified levels of PEG in choroid plexus were more variable than for kidney and liver, due to the small size of the tissue affecting accuracy in measurements.
Nine rats received a single IV administration of 1.5 mg/kg ^{3H}N9-GP (27 MBq/kg, corresponding to ~0.9 mg protein/kg or ~127 IU/kg and PEG-dose of ~ 0.6 mg/kg.). At time-points 1 hour, 12 hours, 24 hours, 4 days, 7 days, 14 days, 35 days, 63 days and 84 days post dose, animals (n=1 per time point) were euthanized and carcasses were prepared for Quantitative Whole Body Autoradiography analysis. Radioactivity in plasma, kidney, choroid plexus and liver was quantified using a validated image analysis system.

Figure 4–2 Decreasing PEG concentrations (pmol/g) and resulting terminal elimination regression lines in plasma, liver, kidney and choroid plexus following administration of N9-GP

For rat plasma, kidney, liver and choroid plexus, PEG elimination half-lives were estimated to 15, 16, 16 and 49 days, respectively. Time to steady-state was then calculated based on the elimination half-life.[52] In rats, steady-state levels in liver, kidney and choroid plexus were predicted to have been reached within 8, 8 and 23 weeks of dosing, respectively (Table 4–2).

Steady-state in all tissues was reached in the 26-week repeat-dose toxicity study in rats where no toxicity was observed.

Using allometric scaling, elimination half-lives in rats were used to estimate the half-lives and time to steady-state in humans (Table 4–2).

Table 4–2 Terminal half-life and time to steady-state estimates for PEG in humans based on terminal half-life data from rat

		Plasma	Liver	Kidney	Choroid plexus
Terminal $t_{½}$	Rat (days)*	15	16	16	49
	Human (days)**	**59**	**63**	**63**	**192**
Time to steady-state***	Rat (weeks)	7	8	8	23
	Human (months)	**6**	**7**	**7**	**21**

Body weight (BW) for human: 70 kg; BW for rat: 0.3 kg.
* Observed data based on regression lines from terminal elimination phase
** Human $t_{½}$ = rat $t_{½}$ x (BW human/BW rat)^0.25.[52]
*** 3.3 x $t_{½}$ (90% steady-state).[52]

Time to steady-state was predicted to be within 6 months to 2 years for human plasma and tissues. This is important as patients have been treated with N9-GP for more than four years in the N9-GP clinical program with no unexpected adverse effects (Table 7–1).

4.5 Plasma tissue model for PEG distribution and elimination

Plasma is the central compartment from where PEG is distributed to and from tissues (Figure 4–3). In tissues, the rate of input is determined by the plasma concentration, and the rate of output is determined by the tissue elimination rate (as determined in the rat distribution study, see Section 4.4. Steady state in tissues is reached when there is equilibrium between the input and the output rates.

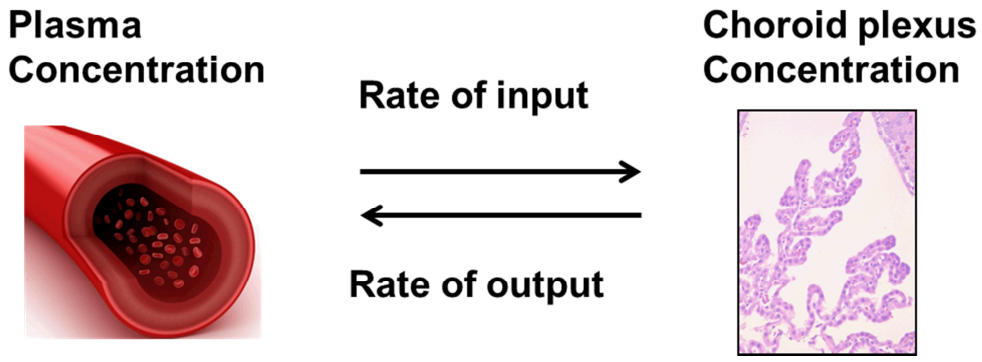


Figure 4–3 Tissue steady-state is reached when there is equilibrium between rate of input from plasma and rate of output, i.e., elimination from the tissue

The rat distribution data were used to build a plasma tissue model to simultaneously fit the observed data. The following general assumptions were used in the modelling approach:

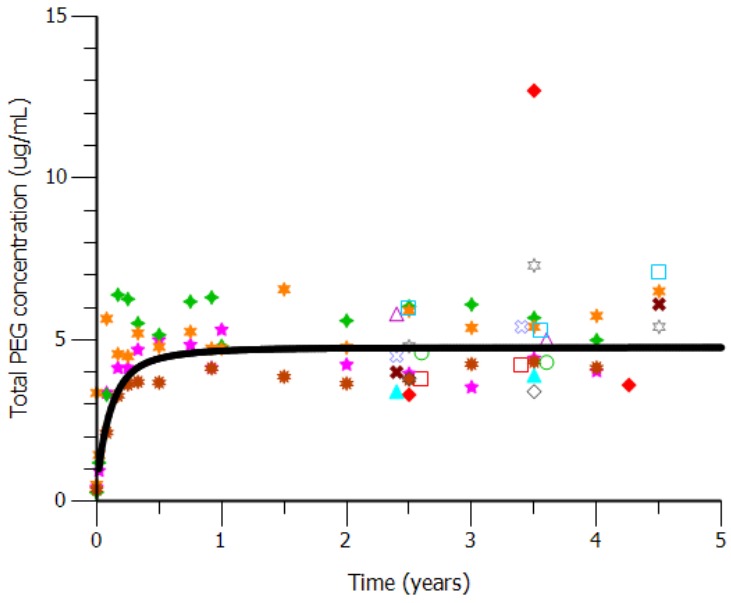
- The kinetics of PEG is dose-linear
- The kinetics of PEG is constant over time

- Kinetic model parameters can be scaled using simple allometric scaling

This model could adequately capture the plasma and tissue data for rats and was used to simulate human steady-state plasma and tissue PEG concentrations with a once-weekly 40 IU/kg N9-GP dose.

4.6 Measured plasma levels of PEG in pediatric patients

In humans, PEG concentrations were quantified by Nuclear Magnetic Resonance (^{1}H-NMR) in plasma samples from patients in the ongoing extension phase of Trial 3774, where pediatric patients are treated with 40 IU/kg N9-GP once-weekly prophylaxis. As shown in Figure 4–4, steady-state PEG plasma levels were achieved and were similar to the levels predicted by the plasma-tissue model.



Nuclear Magnetic Resonance (^{1}H-NMR) was used to quantify the total PEG concentration (i.e., as conjugated to N9-GP or as free PEG) in human plasma samples collected during treatment for up to 4.5 years with 40 IU/kg once-weekly N9-GP in children. The numbers of measurements are between 4 and 14 per time point.

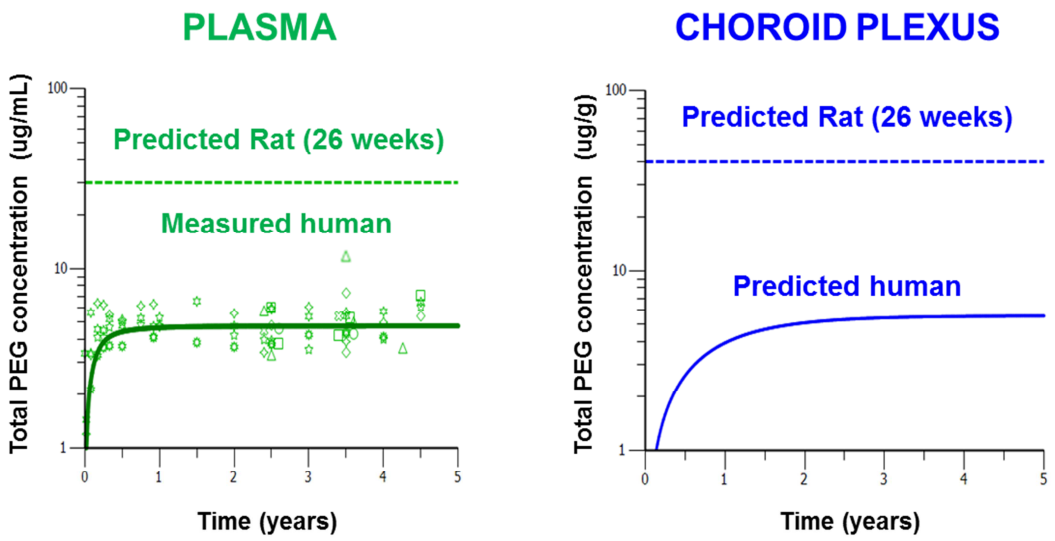
Each unique symbol represents measurements in one individual patient at different time points (14 different pediatric patients in total).

Solid black line represents the predicted human steady-state PEG concentrations from the rat applying allometric scaling factor of 0.67 for clearance.

Figure 4–4 Measured (symbols) and modeled (solid black line) PEG plasma concentration at steady-state in the pediatric trial (Trial 3774) during once-weekly treatment with 40 IU/kg N9-GP for up to ~4.5 years

One pediatric patient (indicated with red diamond symbols in the figure) had a higher total PEG concentration in plasma at the 3.5 year time point compared to the 2.5 year time point; this is consistent with this patient receiving a total of 35 infusions with 40 IU/kg N9-GP for treatment of bleeds within the 4.5 months prior to collecting the 3.5 year plasma sample. Steady-state prediction is based on once weekly 40 IU/kg dose which would correspond to 18 infusions in 4.5 month. At the 4.5 years sample, this patient had returned to a PEG concentration close to the predicted steady-state levels after resuming his normal prophylactic treatment schedule.

Human steady-state PEG levels in choroid plexus were estimated to be 7 times lower than the PEG concentrations reached in the 26-week rat study at the highest N9-GP dose, where no PEG-related vacuolation or other adverse effects were observed (1200 IU/kg/5d, or 9660 µg PEG/kg/week) (Figure 4–5).



Human dose: once-weekly 40 IU/kg N9-GP (~230 µg PEG/kg/week), Rat dose: 1200 IU/kg/5d N9-GP (~ 9660 µg PEG/kg/week)

Figure 4–5 Measured concentrations of PEG in human plasma (green symbols) and predicted steady-state concentrations in plasma and choroid plexus (solid lines) compared to the 26-week rat study (dashed lines) after N9-GP dosing

Acknowledging the limitations of using modeling data, the actual plasma PEG concentrations observed in pediatric patients provide reassurance that PEG steady state concentrations will be reached at levels well below a PEG concentration level where no toxicities, no PEG-related vacuolation, and no cellular changes have been observed in the chronic toxicity study in rat. In the clinical program, 62 patients have been exposed to N9-GP for more than 2 years, 22 being children (0-12 years) in the extension phase of the pediatric trial (see Table 1–2).

4.7 Non-clinical data from other PEGylated protein therapeutics

PEG is widely used in food products, cosmetics and pharmaceuticals (either as excipient or conjugated to the active drug). For example PEG (3 kDa) has been used as an excipient in the anti-hemophilic factor Hemofil M since 1966. An extensive literature review substantiates that at clinically relevant doses, PEG is inert and there are no functional changes or changes of toxicological relevance.[6, 8, 9, 14, 51] PEGylation is a well-established technique commonly used to prolong circulating half-life of drugs. A large number of PEGylated products are on the market and in development and the nonclinical profile of PEG has been extensively characterised.[6, 8-14] A recent review by Ivens et al., 2015[14] summarize the nonclinical safety profile of PEGylated therapeutics in development or approved for marketing:

- 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.
- Cellular vacuolation attributed to the PEG moiety of different sizes has been observed in some toxicology studies, primarily in macrophages in various tissues.
- For larger PEG molecules, especially 40 kDa PEGylated proteins, vacuolation was also seen in the choroid plexus epithelial cells at very high doses.
- The occurrence, incidence and severity of tissue vacuolation have been described to be related to dose, dose frequency and treatment duration.
- The presence of PEG-related vacuoles did not result in any cellular reaction, e.g., no inflammation, degeneration or necrosis.
- Reversibility of PEG-related vacuolation depends on elimination half-life of the PEG moiety and rate of cellular turnover.

Novo Nordisk also conducted toxicology studies with very high doses of 40 kDa PEG alone, which confirmed the findings reported from some approved drugs; i.e., vacuole formation in macrophages and in the choroid plexus epithelial cells (see Appendix I in Section 11).

Based on the combined evidence, including significant literature data, PEG is inert and does not cause functional changes or changes of toxicological relevance at the proposed N9-GP clinical doses. Furthermore, in cases where vacuolation has been seen non-clinically, there is no *in vivo* animal data relating vacuole formation to functional consequences at clinically relevant doses.

4.8 Clinical data from other PEGylated therapeutics

The clinical experience with PEGylated proteins is known from 12 PEGylated biopharmaceutical products currently approved in the US for episodic or chronic treatment of various conditions (Table 4–3). The weekly clinical PEG exposure levels resulting from these products ranges from 0.1 to 1,000 µg/kg/week and the PEG molecular size ranges from 5 to 40 kDa. For comparison, a once-weekly 40 IU/kg dose of N9-GP corresponds to ~230 µg/kg/week 40 kDa PEG.

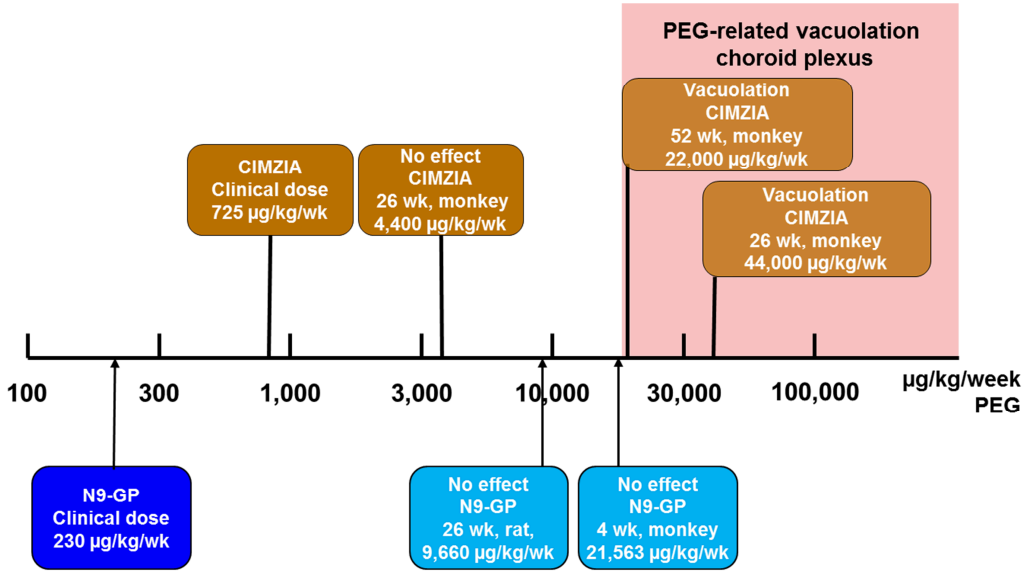
Table 4–3 N9-GP and US approved PEGylated biopharmaceutical products

Product	PEG Size	Typical dose	Clinical PEG exposure per week [(1)]	Indication	US Approval
N9-GP	40 kDa	2400 IU [(2)]	230 µg/kg	Hemophilia B	NA
Cimzia®	40 kDa	400 mg	725 µg/kg	CD, RA, PsA, AS[(3)]	2008
Pegasys®	40 kDa	0.18 mg	2 µg/kg	Hepatitis C & B	2002
Macugen®	40 kDa	0.3 mg	0.7 µg/kg	AMD	2006
Mircera®	30 kDa	0.036 mg [(2)]	0.1 µg/kg	Renal anemia	2007
Neulasta®	20 kDa	6 mg	33 µg/kg	Neutropenia	2002
Plegridy®	20 kDa	125 µg	1 µg/kg	Multiple Sclerosis	2014
Adynovate®	20 kDa	4.2 mg [(2)]	15 µg/kg	Hemophilia A	2015
PegIntron®	12 kDa	0.064 mg [(2)]	0.4 µg/kg	Hepatitis C	2000
Krystexxa®	10 kDa	8 mg	12,000	Chronic gout	2010
Adagen®	5 kDa	1200 IU [(2)]	Not available	Immunodeficiency	1990
Oncaspar®	5 kDa	4000 IU [(2)] (~47 mg)	1000 µg/kg	Leukemia	2016
Somavert®	5 kDa	10 mg	980 µg/kg	Acromegaly	2002

AMD: Age-related macular degeneration.
The list is adopted and modified from Ivens et. al., 2015[14] and Stidl et. al., 2016.[9]
[(1)] Weekly PEG exposure is calculated based on adult with 60 kg body weight[9] ; [(2)] Dose is based on a 60 kg person ; [(3)] 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.

In relation to the PEG size, clinical PEG exposure, and duration of treatment, a relevant comparator to N9-GP is Cimzia® (certolizumab pegol). It is a 40 kDa PEGylated anti-TNFα pharmaceutical product that has been marketed since 2008 for the treatment of Crohn's disease in adults. When administered according to label, the weekly PEG exposure from Cimzia® is approximately 725 µg/week, where the PEG exposure from once weekly N9-GP prophylaxis is 230 µg/week. Consistent with this higher dose, PEG concentrations measured in pregnant women treated with Cimzia were ~11-75 µg/mL (mean 35.8 µg/mL measured 5-42 days after Cimzia dosing, N=8).[53] In comparison, steady-state plasma PEG concentrations were ~3-7 µg/mL in children on once weekly N9-GP prophylaxis (mean= 4.8 µg/mL measured one week after N9-GP dosing, n=14).

In contrast to N9-GP, cellular vacuolation attributed to the PEG moiety was observed in non-clinical toxicology studies with Cimzia®, both in macrophages in various tissues and also in the choroid plexus epithelial cells at PEG doses of approximately 22,000 µg/kg/week (Figure 4–6).



Modified from Ivens et al., 2013[8]

Figure 4–6 Clinical and non-clinical weekly exposure levels of PEG from treatment with Cimzia® (brown boxes) and N9-GP (blue boxes).

A Cochrane review[54] including 163 controlled and 46 open-label trials compared the adverse events reported following treatment with Cimzia® to those reported for four other available non-PEG-containing anti-TNFα therapies (etanercept, infliximab, adalimumab and golimumab). The assessment concluded that Cimzia® did not differ significantly from the four other anti-TNF agents in the rate of total adverse events or the rate of patient withdrawals due to adverse events. Also, the majority of adverse events reported during treatment with Cimzia® were mild or moderate and were related to the effects of TNFα, and not to PEG.[54, 55]

To date, there are no reported safety concerns with regards to PEG accumulation arising from post-marketing data from PEGylated products, including products approved for chronic use. Thus, more than 20 years of post-authorization experience support the long-term clinical safety of currently marketed PEGylated products.

4.9 Conclusion

There are no nonclinical or clinical data for N9-GP substantiating the theoretical safety concern regarding PEG accumulation; however, the Risk Management Plan includes collection of additional N9-GP long-term clinical safety and exposure data (Section 8).

5 Clinical pharmacology

Summary

- The half-life of N9-GP was shown to be approximately 5 times longer than recombinant FIX (BeneFIX®) and plasma derived FIX in a head-to-head comparison in the Phase 1 trial.
- The steady-state terminal half-life of N9-GP 40 IU/kg was ~111 hours.
- The significantly prolonged half-life ($t_{½}$) and increased incremental recovery during 40 IU/kg prophylaxis with N9-GP resulted in high sustained FIX activity levels with a simplified once-weekly dosing schedule.
    - Pre-dose or trough FIX levels were 27.3% in adolescents/adults, 19% in children aged 7-12 years, and 15% in children aged 0-6 years.
    - FIX activity levels were in the non-hemophilia range (>40%) for approximately 5.4 days per week in adults and adolescents, and approximately 2.3 days per week in children.
- There were no apparent differences in the pharmacokinetic parameters across races, or between normal-weight compared to overweight patients.

5.1 Methodology

The FIX activity is considered pharmacodynamic in nature and reflects the biologic activity of any factor IX product, including N9-GP. No other pharmacodynamic endpoints have been found applicable to assess in the clinical development program for N9-GP. The pharmacokinetic properties of N9-GP were based on pharmacodynamic measurements of FIX activity, which generally is considered as a surrogate marker for clinical efficacy of FIX products.

5.1.1 Analytical methods

The primary assay for assessment of FIX activity in the completed clinical trials was the one-stage clotting assay using N9-GP product-specific standard (PSS) as calibrator. The analyses were performed at a central laboratory. The aPTT reagent was changed from TriniCLOT in phase 1 to SynthAFax in phase 3 (Table 5–1). Following validation of SynthAFax with normal human plasma (NHP) as calibrator, the one-stage clotting assay with NHP calibration is used in the ongoing trial in previously untreated patients (Trial 3895).

In addition, FIX activity was measured using the chromogenic assay with NHP as calibrator at Novo Nordisk, Denmark. The chromogenic assay is based on color development by FXa cleavage of a chromogenic substrate.

Table 5–1 Assays used in N9-GP clinical trials

	One-stage Clot Assay		Chromogenic FIX
	aPTT reagent	**Calibrator**	**Calibrator**
Trial 3639 Phase 1 PK	TriniCLOT aPTT (Stago)	PSS	ND
Trial 3747 Phase 3 adult/adolescent PTP	SynthAFax (IL)	PSS	NHP
Trial 3775 Phase 3b Extension PTP	SynthAFax (IL)	PSS	ND
Trial 3773 Phase 3b Surgery	SynthAFax (IL)	PSS	ND
Trial 3774 Phase 3 pediatric – PTP	SynthAFax (IL)	PSS	NHP
Trial 3895 Phase 3 pediatric – PUP	SynthAFax (IL)	PSS and NHP	NHP

PSS= N9-GP product-specific standard, PTP= Previously treated patient, PUP= Previously untreated patient, NHP= Normal human plasma, ND= Not done, IL=Instrument Laboratory

The results from the one-stage clotting assay and the chromogenic assay were compared in the pivotal trial (Trial 3747) and the pediatric trial (Trial 3774). A high correlation between the results from the two assays was obtained. The ratio of FIX activity levels between chromogenic and one-stage clot assays was approximately 1 in 42 patients (17 adult/adolescent, 25 children) (Figure 5–1).

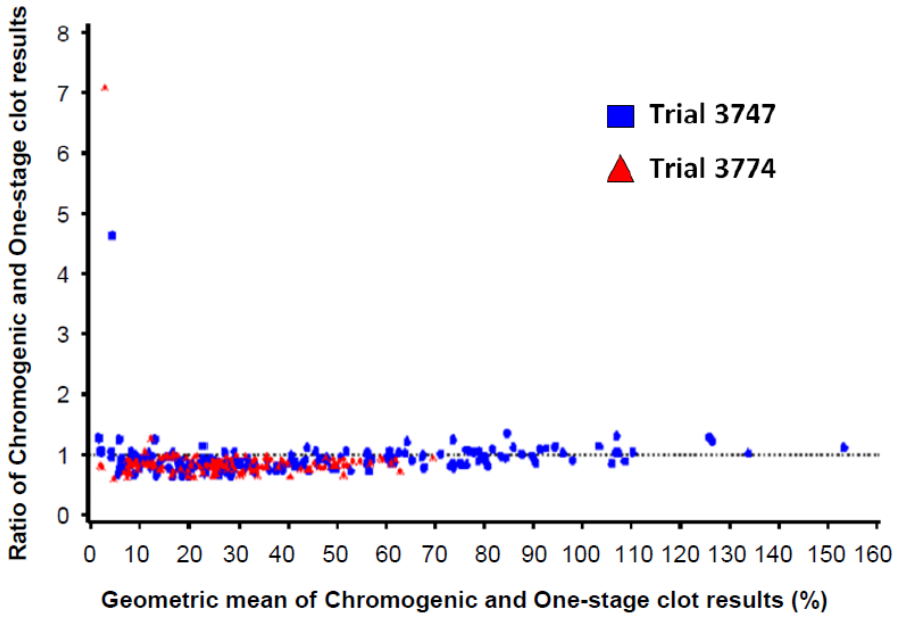


Figure 5–1 Bland-Altman plot of FIX activity obtained with one-stage clot (PSS) and chromogenic assay (NHP)

5.2 Trial designs

In Trial 3639 the pharmacokinetic profile of N9-GP was investigated and compared to patients' previous FIX product (plasma-derived FIX [pdFIX] or recombinant FIX [rFIX]) at three different dose levels (25, 50 and 100 IU/kg).

Single-dose pharmacokinetics of N9-GP was assessed in a total of 52 patients: 15 in phase 1 (Trial 3639), a subset of 13 adolescents/adults in the pivotal study (Trial 3747) and 25 pediatric patients (0−12 years) in the pediatric trial (Trial 3774) (Table 5–2).

Steady-state pharmacokinetics (i.e., after 3-6 months once-weekly treatment) was assessed in 16 adolescent/adult patients in Trial 3747 (9 patients receiving 40 IU/kg, 7 patients receiving 10 IU/kg) (Table 5–2).

PK samples were collected prior to dosing and at multiple time points up to 168 hours after dosing.

Table 5–2 Pharmacokinetic assessments in clinical development (Trials 3639, 3747, 3774)

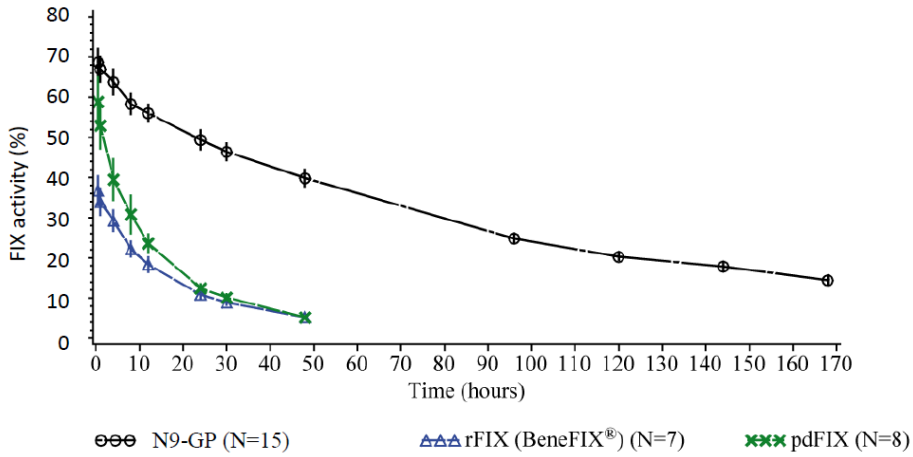
Trial	Patients with Single-Dose PK N (dose)	Patients with Steady-State PK N (dose)
Trial 3639 Phase 1 PK	5 (25 IU/kg) 5 (50 IU/kg) 5 (100 IU/kg)	None
Trial 3747[a] Phase 3 adult/adolescent PTP	4 (10 IU/kg) 9 (40 IU/kg)	7 (10 IU/kg) 9 (40 IU/kg)
Trial 3774 Phase 3 pediatric PTP	25 (40 IU/kg)	None

[a] 4 patients from Trial 3639 also participated in Trial 3747 PK assessment, where 1 of these patients had single dose and steady state assessment in 3747 and 3 patients had only steady state assessment on 10 IU/kg.

5.3 Pharmacokinetics

5.3.1 Single-dose pharmacokinetics in adult and adolescent patients

Dose linearity for N9-GP was evaluated based on AUC and $C_{30\ min}$ at three dose levels (25, 50 and 100 IU/kg) in phase 1 (Trial 3639), and the results indicated dose linearity within the tested dose-range. Additionally, the pharmacokinetics of patients' previous FIX product (pdFIX, rFIX [BeneFIX®]) was compared to N9-GP in phase 1 (Trial 3639). The mean terminal half-life of N9-GP was 93 hours. This was 5-fold longer than the patients' previous FIX products (Figure 5–2).



rFIX = recombinant factor IX; pdFIX = plasma derived FIX

Figure 5–2 Mean single-dose pharmacokinetic profiles of N9-GP and patients' previous FIX product (pdFIX and rFIX) dose normalized to 50 IU/kg (Trial 3639)

Single dose pharmacokinetics of N9-GP in 13 adults and adolescents of the pivotal phase 3 study (Trial 3747) revealed similar half-life and recovery (Table 5–2).

5.3.2 Single-dose pharmacokinetics in pediatric patients

In children, compared to adults and adolescents, the incremental recovery was lower and the body weight-adjusted clearance was higher, which resulted in overall lower FIX activity levels in the pharmacokinetic profiles. The trend of increasing incremental recovery and decreasing clearance (mL/h/kg) with age (Table 5–3) is comparable to what has been observed for other FIX products.[20, 21, 56-59]

Table 5–3 Summary of single-dose pharmacokinetic parameters by age (one-stage clotting assay) - 40 IU/kg N9-GP (Trials 3747 and 3774)

Parameter	0−6 years	7−12 years	13−17 years	18−70 years
Incremental Recovery (%/[IU/kg])				
N	11[a]	13	3	6
Geometric Mean (CV%)	1.5 (7.3)	1.6 (16.18)	2.0 (14.74)	2.3 (11.32)
Median	1.5	1.6	1.9	2.3
Min ; Max	1.3, 1.7	1.2, 2.3	1.8; 2.3	2.1; 2.9
Clearance (mL/h/kg)				
N	12	13	3	6
Geometric Mean (CV%)	0.8 (13.0)	0.6 (21.9)	0.5 (30.4)	0.4 (14.7)
Median	0.8	0.7	0.4	0.4
Min ; Max	0.6; 1.0	0.4; 1.0	0.4; 0.7	0.3; 0.5
Terminal half-life, t½ (h)				
N	12	13	3	6
Geometric Mean (CV%)	69.6 (15.8)	76.3 (25.5)	89.4 (24.1)	83.0 (22.5)
Median	72.1	76.6	96.0	83.7
Min ; Max	45.1; 81.7	49.9; 150.2	68.6; 108.6	55.8; 107.0
FIX activity 168h post dosing (%)				
N	11[b]	12[b]	2[b]	6
Geometric Mean (CV%)	8.4 (16.28)	10.9 (18.89)	14.6 (59.64)	16.8 (30.61)
Median	8.6	10.6	15.8	17.4
Min; Max	5.8; 10.7	7.6; 14.1	9.9; 21.6	11.4; 24.8
AUC(0-inf) (IU×h/mL)				
N	12	13	3	6
Geometric Mean (CV%)	46.2 (14.1)	56.2 (19.1)	79.9 (34.7)	90.6 (16.1)
Median	45.5	56.6	92.8	87.1
Min ; Max	36.6; 59.4	39.3; 72.5	54.3; 101.1	78.6; 118.4

CV%: coefficient of variation

[a] For one patient, the pre-dose and 30 min post dose samples were not collected; [b] For one patient, the 168h post dose sample was not available.

5.3.3 Single-dose pharmacokinetics by race and body mass index

There were no apparent differences in the pharmacokinetic parameters across the races White, Black, Asian and others.

Of the 16 patients with pharmacokinetic assessments in the pivotal trial (Trial 3747), 10 patients had a body mass index (BMI) ≥ 25 kg/m^2, and 6 patients were within normal range (BMI 18.5-24.9 kg/m^2) at baseline. There were no apparent differences in the pharmacokinetic profiles of FIX activity between normal-weight and overweight patients.

5.3.4 Steady-state pharmacokinetics

Steady-state pharmacokinetic in a subset of 9 adolescents and adults receiving 40 IU/kg and 7 receiving 10 IU/kg are shown in Figure 5–3.

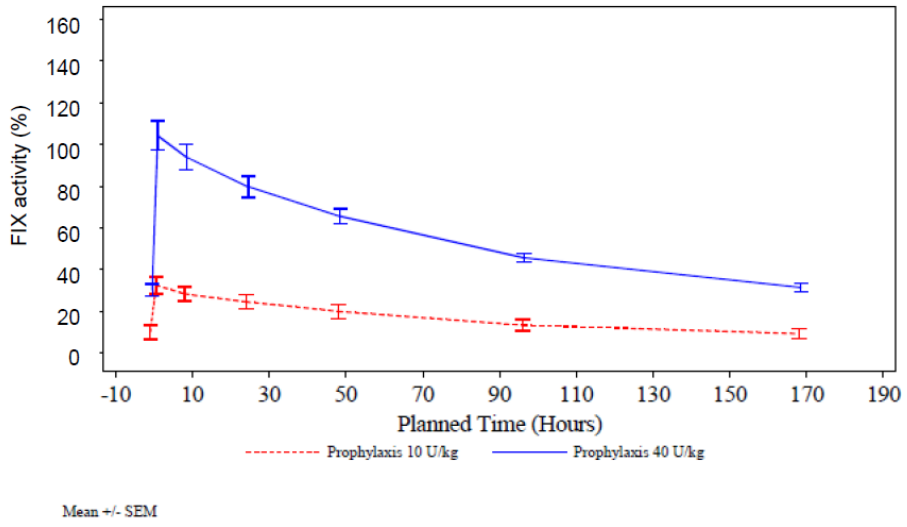


Figure 5–3 Steady-state profiles of FIX activity following administrations of 40 IU/kg and 10 IU/kg N9-GP (Trial 3747)

After intravenous infusion of N9-GP, the plasma FIX activity declined exponentially (one-compartment model), both after single-dose administration and at steady-state.[15] Due to the long half-life of N9-GP some accumulation of FIX activity was observed and pre-dose FIX activity was higher at steady-state compared to single dose. As a consequence, FIX activity for the steady-state pharmacokinetic profile was higher than single-dose. The estimated accumulation ratio was 1.43 for once weekly dosing of 40 IU/kg N9-GP.

Steady state pre-dose (trough) FIX levels were also measured during prophylaxis (Trials 3747, 3774). Results were analyzed using a mixed effect model on log-transformed plasma FIX activity with dose as a fixed effect and patient as a random effect (up to 9 measurements per patient). The estimated mean FIX trough level was presented using estimates back-transformed to the natural scale together with the 95% CI.

With N9-GP 40 IU/kg once-weekly prophylaxis, mean pre-dose (trough) FIX levels were 27.3% in adult and adolescent patients compared with 8.5% with 10 IU/kg once-weekly (Figure 5–4 and Table 5–4).

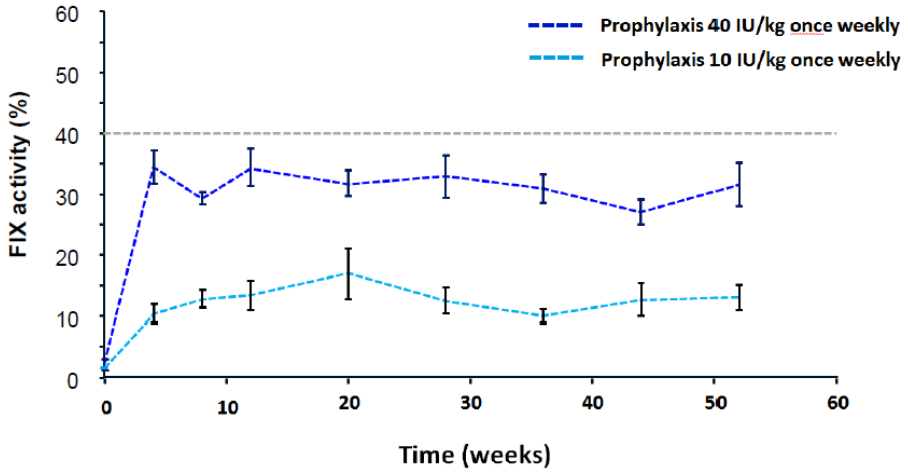


Figure 5–4 Steady-state pre-dose (trough) FIX levels in adults and adolescents (Trial 3747)

Table 5–4 Steady-state pre-dose (trough) and peak FIX levels in adults and adolescents (Trial 3747)

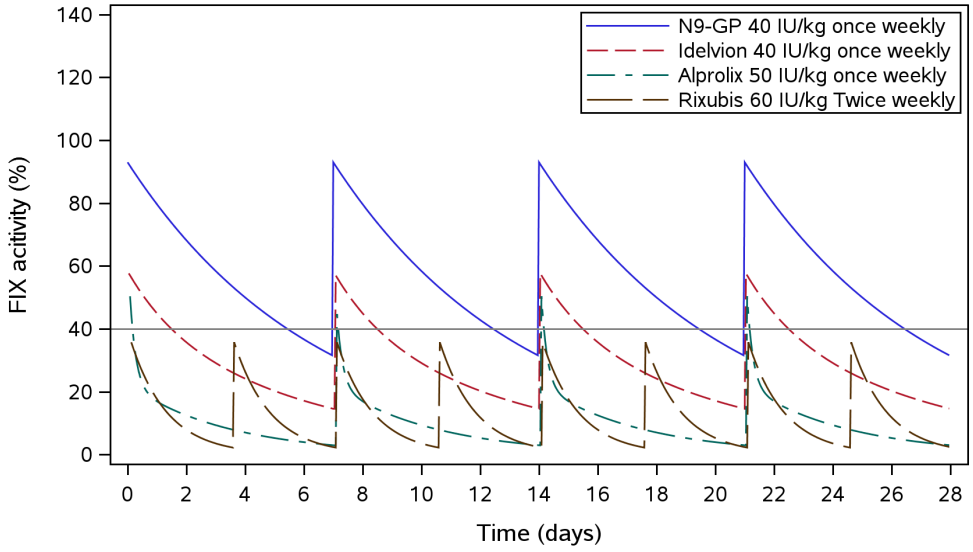
	N9-GP prophylaxis regimen	
	40 IU/kg once-weekly	**10 IU/kg once-weekly**
Number of patients	29	30
Number of pre-dose (trough) values analyzed	194	193
Number of pre-dose (trough) values excluded[a]	55	70
Mean pre-dose (trough) level[b] (%) 95% CI	27.3 24.8, 30.0	8.5 7.7, 9.3
Number of peak values analyzed	166	168
Number of peak values excluded[a]	50	64
Mean peak level[b] (%) 95% CI	92.6 85.5, 1.00	30.2 28.0, 32.7

[a] To be included in the analysis, measurements must have been taken at least 5 days and no more than 10 days after the last dose with a coagulation factor product as well as at least 14 days after last bleeding episode. No values were excluded due to values below lower limits of quantification.
[b] Based upon a mixed model of the log-transformed plasma concentrations with patient as a random effect. The mean trough level is presented back-transformed to the natural scale.

Time from administration of 40 IU/kg to a plasma FIX activity of 40% (i.e., within the non-hemophilia range) was also predicted based on observed steady-state FIX activity profiles using a

one-compartment pharmacokinetic model with first-order elimination and parameters of clearance (CL) and volume of distribution (Vss).[15] After repeated once-weekly 40 IU/kg dosing, an adult or adolescent hemophilia B patient is predicted to have FIX activity above 40% for 130.4 hours (5.4 days), i.e., within the non-hemophilia range for 77.6% of the dosing interval.

The ability of prophylaxis with N9-GP 40 IU/kg once-weekly to achieve non-hemophilia FIX levels was substantially greater than could be achieved with biweekly dosing of current standard or weekly dosing of current extended half-life products (Figure 5–5 and Table 5–5).[16, 17] The FIX levels achieved with 40 IU/kg once-weekly were also greater than can be achieved with dosing current extended half-life FIX products at longer intervals (Figure 5–6 and Table 5–5).



The grey line indicates a FIX activity level of 40%.
The predicted pharmacokinetic profiles are based on PK models according to Tiede et al.[15] (N9-GP), Zhang et. al.[16] (IDELVION®), Powell et al.[17] (ALPROLIX®), and RIXUBIS USPI[18].

Figure 5–5 Predicted steady-state FIX activity profiles with once-weekly dosing of N9-GP, IDELVION® and ALPROLIX® and twice-weekly dosing of RIXUBIS in adults and adolescents

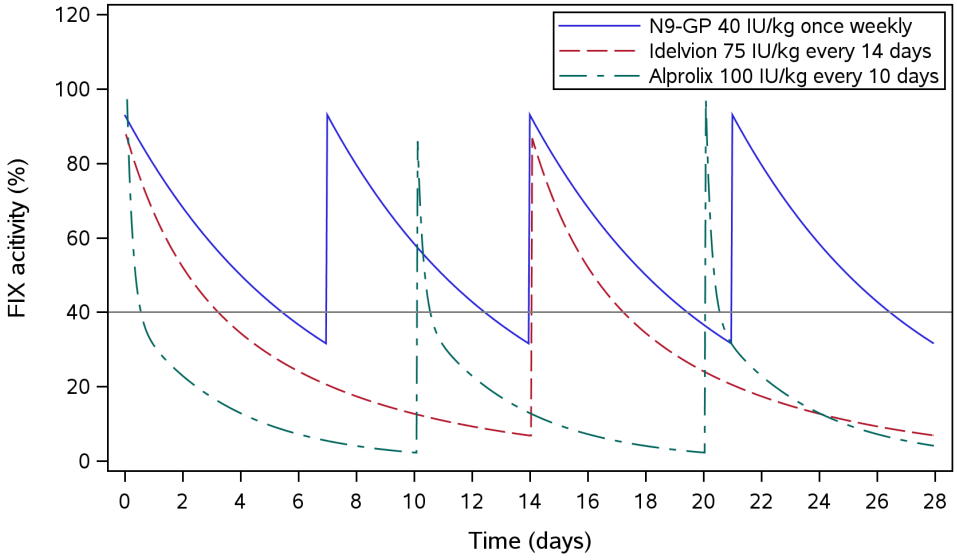


Figure 5–6 Predicted steady-state FIX activity profiles with once-weekly dosing of N9-GP and 10-14 day dosing of IDELVION® and ALPROLIX® in adults and adolescents

Table 5–5 Predicted steady-state FIX activity with N9-GP, IDELVION®, ALPROLIX®, and RIXUBIS in adults and adolescents

Treatment	Days per week above 40% FIX activity	% of week above 40% FIX activity
N9-GP 40 IU/kg once-weekly	5.4	78
N9-GP 10 IU/kg once-weekly	0	0
IDELVION® 40 IU/kg once-weekly	1.4	21
IDELVION® 75 IU/kg every 14 days	3.2	23
ALPROLIX® 50 IU/kg once-weekly	0.1	1
ALPROLIX® 100 IU/kg every 10 days	0.5	5
RIXUBIS 60 IU/kg every 4 days	0	0

In children, steady-state is reached after 3-4 weeks. Steady-state FIX levels were maintained above 15% for the entire treatment period; younger children (ages 0-6) had steady-state pre-dose (trough) levels of 15%, while older children (ages 7-12) had pre-dose trough levels of 19% (Figure 5–7).

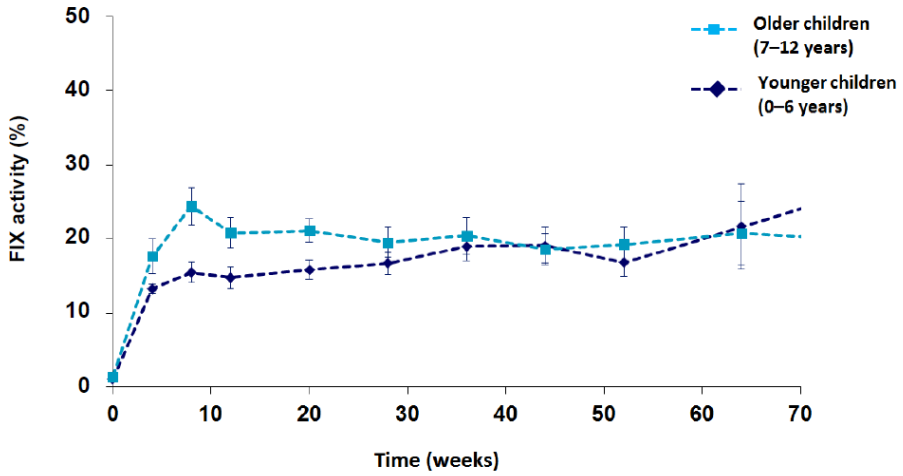


Figure 5–7 Steady-state pre-dose (trough) FIX activity in children (Trial 3774)

Table 5–6 Steady-state pre-dose (trough) and Peak FIX activity in children (Trial 3774)

	N9-GP 40 IU/kg Once-Weekly Prophylaxis		
	0 - 6 years	**7 - 12 years**	**Total**
Number of patients	12	13	25
Number of pre-dose trough values analyzed	87	100	187
Number of pre-dose trough values excluded	3	9	12
Mean pre-dose trough level[a] (%) 95% CI	15.4 12.7, 18.6	19.0 15.9, 22.8	17.2 15.0, 19.7
Number of peak values analyzed	78	79	157
Number of peak values excluded	2	9	11
Mean peak level[a] (%) 95% CI	65.5 60.6, 70.7	71.4 66.3, 77.0	68.5 64.8, 72.4

[a] Based upon a mixed model of the log-transformed plasma concentrations with patient as a random effect. The mean through level is presented back-transformed to the natural scale.

While more rapid metabolism of all factor products in children makes it more difficult to achieve or sustain higher levels of factor VIII or IX, children as a whole on N9-GP were able to achieve levels in the non-hemophilia range for 2.3 days per week or 33% of the week (Table 5–7). Modeling of N9-GP compared with other currently approved extended half-life products suggests that N9-GP is able to achieve levels in the non-hemophilia range for twice the period of IDELVION®; ALPROLIX® was unable to achieve non-hemophilia FIX levels (Table 5–7).

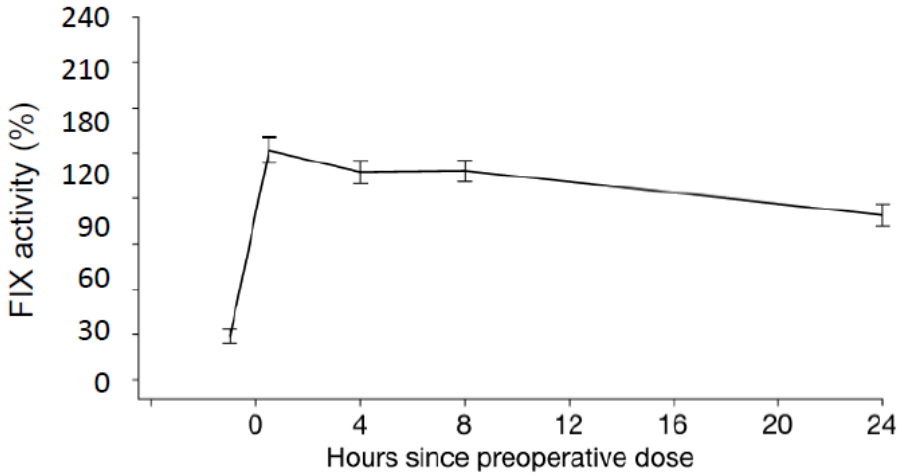
Table 5–7 Predicted steady-state FIX activity with N9-GP, IDELVION®, and ALPROLIX® in children

Treatment	Days per week above 40% FIX activity	% of week above 40% FIX activity
N9-GP 40 IU/kg once-weekly	2.3	32.5
IDELVION® 50 IU/kg once-weekly*	1.1	15.1
ALPROLIX® 60 IU/kg once-weekly*	0.0	0.0

* Non-compartmental model based upon labeled data for IDELVION® and ALPROLIX®

5.3.5 FIX activity during surgery

The FIX activity following an initial 80 IU/kg N9-GP infusion prior to major surgery is shown in Figure 5–8. Patients were covered by median FIX activity levels above 100% for at least 24 hours on average (Table 5–8).



Copied from Escobar et. al from 2016[60]

Figure 5–8 Mean FIX activity (± SEM) over time from the day of surgery to 24 hours after the preoperative dose.

Table 5–8 FIX activity following initial 80 IU/kg pre-operative infusion in the surgery trial (Trial 3773)

	30 minutes N=13	8 hours N=12[a]	24 hours N=13[a]	48 hours N=7[b]
FIX activity (%) Median (Range)	143 (123-224)	138 (101-175)	112 (62-146)	73 (40-110)

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

6 Clinical efficacy

Summary

- **Development Program:** Efficacy was assessed in 105 previously treated children, adolescents and adults across 4 pivotal/extension trials. Important inclusion criteria in phase 3 trials were patients with hemophilia B and a FIX activity level ≤2% and no history of FIX inhibitors.
- **Primary Endpoint:** The primary endpoint of rate of inhibitor development for 3 studies was met with no inhibitors reported in previously treated patients. For the perioperative management study the primary endpoint of surgical hemostasis was met.
- **Treatment of bleeds**: N9-GP bleed treatment with 40 IU/kg doses was successful in 93.2% of 591 bleeds without dose adjustment.
    - For adolescents/adults receiving 40 IU/kg once-weekly prophylaxis, 97.1% of 69 bleeds were successfully treated and 99% of bleeds were treated with one dose.
    - For children ages 0-12, 92.9% of bleeds were successfully treated with N9-GP.
- **Prevention of Bleeds**: N9-GP resulted in low annualized bleeding rates with a simple once-weekly treatment regimen and no dose adjustment.
    - Patients treated with 40 IU/kg once-weekly prophylaxis had low median ABR in adolescents/adults (1.04) and children (1.00).
    - For adolescents/adults, patients randomized to 10 IU/kg (vs 40 IU/kg) had a significantly higher ABR (median 2.93) and were 2.7 times more likely to have a spontaneous bleed.
- **Target Joints**: Patients receiving 40 IU/kg once-weekly were more likely to have no bleeding in target joints (40 vs 10 IU/kg: 67% vs 8%).
    - Adolescents/adults receiving 40 IU/kg were more likely to have target joints resolved (90%) than those treated with 10 IU/kg (58%).
    - Two children with two target joints had resolution of their target joints.
- **Quality of Life**: Prophylaxis with 40 IU/kg once-weekly positively impacted health-related quality of life, including for adolescents/adults improvement in HAEM-A-QoL and/or EQ5D.
- **Perioperative Management:** N9-GP provided effective hemostasis for 16 major and 35 minor procedures across the clinical trials.
    - The intraoperative hemostatic effect of N9-GP during 13 major surgeries was 100% with fewer post-operative infusions (median 4.0) than with current FIX products; 3 additional major surgeries were performed successfully in the extension trials.
    - Most minor procedures were done with a single pre-operative dose which provided effective hemostasis.

6.1 The clinical development program for N9-GP

6.1.1 Overview of development program

Novo Nordisk is seeking an indication for use in adults and children with hemophilia B for control and prevention of bleeding episodes, perioperative management and routine prophylaxis.

Four trials in previously treated adolescent and adult patients (Trials 3639, 3747, 3773, and 3775) and one trial in previously treated pediatric patients (Trial 3774 [main phase]) have been completed. An extension phase of Trial 3774 in previously treated pediatric patients (Trial 3774) is still ongoing. In addition, a trial in previously untreated patients (<6 years) is ongoing (Trial 3895).

An overview of completed and ongoing trials is provided in Table 6–1.

Table 6–1 Overview of clinical trials with N9-GP

Trial ID Phase Status	Trial design	Treatment[a]	Number of dosed patients[b] and actual age ranges	Primary endpoint
Previously treated patients				
Trial 3639 Phase 1 Completed	*First human dose trial* A multicentre, open-label, dose escalation trial evaluating safety and pharmacokinetics of three ascending single doses (25, 50 and 100 IU/kg).	25, 50 or 100 IU/kg (N9-GP, single dose) 25, 50 or 100 IU/kg (previous FIX product, single dose)	Total: 16 adult patients (21-55 years). Pharmacokinetics: 15 patients.	Safety parameters: adverse events, medical events of special interests, antibody formation and inhibitors, physical examination, vital signs, ECG, clinical laboratory assessments and infusion site reactions.
Trial 3747 Phase 3 Completed	*Pivotal trial* A multicentre, single-blind[c], non-controlled, randomized trial evaluating safety, efficacy and pharmacokinetics in routine prophylaxis and treatment of bleeds. Three treatment arms; 10 IU/kg or 40 IU/kg once-weekly prophylaxis for 52 weeks (randomized), or on-demand treatment for 28 weeks.	*Prophylaxis*: 10 IU/kg or 40 IU/kg once-weekly. *Treatment of bleeds*: Mild and moderate bleeds were treated with infusion(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	Total: 74 adolescent or adult patients (13-65 years). Prophylaxis: 59 patients. On-demand: 15 patients. Pharmacokinetics (3 batches): 16 patients.	Incidence of inhibitory antibodies against FIX defined as titer ≥0.6 BU.
Trial 3775 Phase 3 Completed	*Extension trial to Trials 3747 and 3773* A multicentre, open label, non-controlled trial evaluating long-term safety and efficacy in routine prophylaxis and treatment of bleeds. Four treatment arms; 10 IU/kg or 40 IU/kg once-weekly prophylaxis, 80 IU/kg once every second week prophylaxis, or on-demand treatment. Free choice between available treatment arms, and switching of treatment arm during the trial was allowed.	*Prophylaxis:* 10 IU/kg or 40 IU/kg once-weekly, or 80 IU/kg once every second week. *Treatment of bleeds*: Mild and moderate bleeds were treated with infusion(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	Total: 71 adolescent or adult patients (14-66 years). Prophylaxis at baseline: 66 patients. On-demand at baseline: 5 patients.	Incidence of inhibitory antibodies against FIX defined as titer ≥0.6 BU.

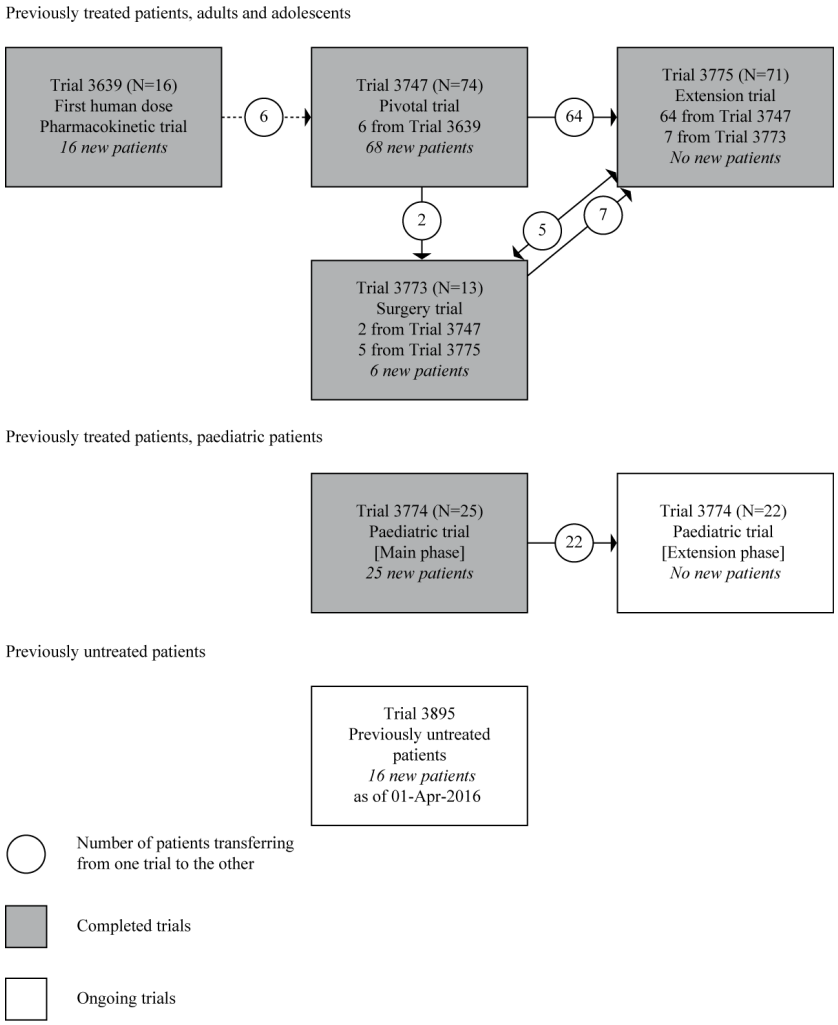
Trial ID Phase Status	Trial design	Treatment[a]	Number of dosed patients[b] and actual age ranges	Primary endpoint
Previously treated patients (continued)				
Trial 3773 Phase 3 Completed	*Surgery trial* A multicentre, open-label, non-controlled trial evaluating efficacy and safety during major surgical procedures.	*Pre-operative dose:* 80 IU/kg on the day of surgery. *Post-operative dose(s):* Recommended to give 2 doses of 40 IU/kg within the first 6 days after surgery.	Total: 13 adolescent or adult patients (15-56 years).	Hemostatic effect during surgery evaluated on a four-point response scale.
Trial 3774 Phase 3 Main phase completed; extension phase ongoing	*Pediatric trial* A multicentre, open-label, non-controlled trial evaluating safety, efficacy and pharmacokinetics in routine prophylaxis and treatment of breakthrough bleeds. The trial contained a main phase of 52 weeks, followed by an extension phase. One treatment arm with 40 IU/kg once-weekly prophylaxis.	*Prophylaxis:* 40 IU/kg once-weekly. *Treatment of bleeds*: Mild and moderate bleeds were treated with infusion(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	Total: 25 pediatric patients (1-12 years). Pharmacokinetics: 25 patients. 22 patients continued into the extension phase of the trial.	Incidence of inhibitory antibodies against FIX defined as titer ≥0.6 BU.
Previously untreated patients				
Trial 3895 Phase 3 Ongoing	*Trial in previously untreated patients* A multicentre, open label, single-arm, non-controlled trial evaluating safety and efficacy of N9-GP in prophylaxis and treatment of breakthrough bleeds in previously untreated patients.	*Prophylaxis:* 40 IU/kg once-weekly. *Treatment of bleeds*: Mild and moderate bleeds were treated with infusion(s) of 40 IU/kg; severe bleeds were treated with 80 IU/kg.	The planned numbers to be exposed is 50 pediatric patients (<6 years)	Incidence of inhibitory antibodies against FIX defined as titer ≥0.6 BU.

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

6.1.2 Number and flow of patients in the clinical trials

A total of 115 unique previously treated patients have been exposed to N9-GP in the 5 completed clinical trials (Trials 3639, 3747, 3773, 3775 and 3774 [main phase]). Excluding the phase 1 pharmacokinetic study (Trial 3639), 105 unique patients have been evaluated for efficacy in the remaining 4 trials.

The majority of the patients participated in more than one trial which is why the sum of patients in the individual trials is higher than the total number of unique patients. The flow of patients in the clinical development program is presented in Figure 6–1.



The figure shows the flow of patients through the completed and ongoing trials in the N9-GP development program. *Numbers in italics* indicate the number of patients exposed to N9-GP for the first time in each trial.

Figure 6–1 Flow of patients in the clinical development program for N9-GP

6.1.3 Trial designs

The phase 3 trials were designed as multinational, multi-center, investigating the safety and efficacy of N9-GP for prophylaxis, treatment of bleeds, and perioperative management, in previously treated patients with severe and moderately-severe hemophilia B and a FIX activity ≤2% according to medical records.

6.1.3.1 Pivotal trial of prophylaxis and on-demand in adolescents and adults (Trial 3747)

As a unique feature of the pivotal and randomized Trial 3747, the trial had two randomized double-blinded prophylaxis arms: 40 IU/kg and 10 IU/kg once-weekly and an on demand arm based upon patient/physician choice (Figure 6–2). The phase 1 pharmacokinetic data (Trial 3689) were used to select the two prophylaxis dose regimens (40 IU/kg and 10 IU/kg once-weekly) for the pivotal trial (Trial 3747) in adolescent and adult patients. The 40 IU/kg prophylaxis regimen was selected to investigate the effect of sustained high FIX activity levels with a less frequent (once-weekly) administration interval. The 10 IU/kg once-weekly prophylaxis regimen was selected to provide a comparison to the effect of FIX levels more consistent with the range of other currently used products.[34, 35, 61-67] Randomization to the two prophylaxis doses allowed for statistical comparison of the efficacy and clinical outcomes between high sustained FIX activity levels (40 IU/kg once-weekly) with levels more consistent with those of current products (10 IU/kg once-weekly).

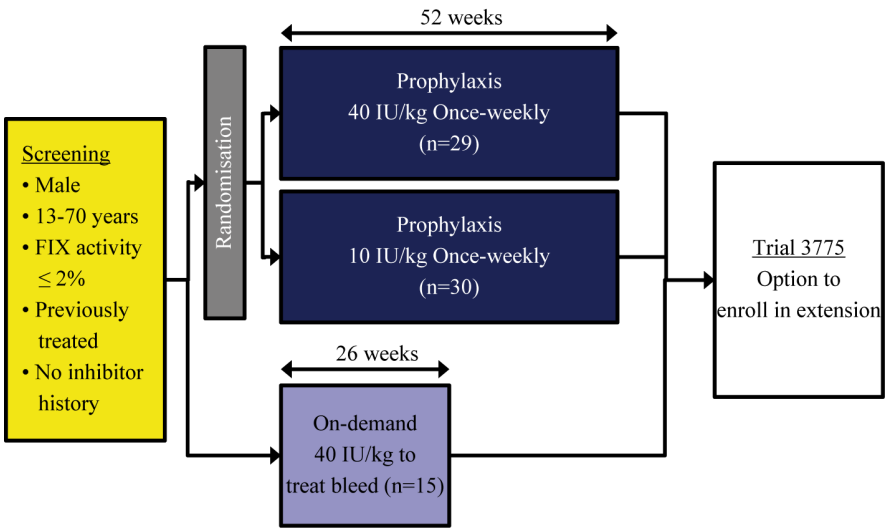


Figure 6–2 Overview of the randomized and blinded Trial 3747

6.1.3.2 Adolescents and adults extension trial (Trial 3775)

The trial was an open label, non-randomized, trial with the purpose of evaluating clinical safety and efficacy for treatment of bleeding episodes and long-term prophylaxis with N9-GP. Patients enrolled in Trial 3775 were recruited from Trial 3747 and Trial 3773 (Figure 6–1).

When entering this extension trial, the patient and the investigator together agreed on a treatment arm. Initially, the choice was between three treatment arms; 10 IU/kg or 40 IU/kg once weekly prophylaxis with N9-GP, or on-demand treatment with 40 IU/kg for bleeding episodes only.

Switching to another treatment arm during the trial was allowed. The trial design is presented in Figure 6–3.

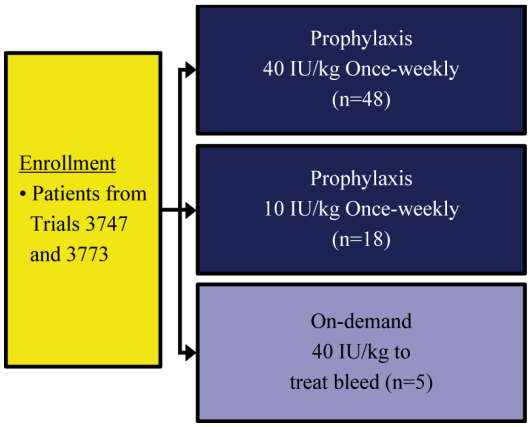


Figure 6–3 Overview of the extension Trial 3775

6.1.3.3 Pediatric trial (Trial 3774)

This was an open-label, confirmatory trial evaluating safety, efficacy and pharmacokinetics of N9-GP in prophylaxis and treatment of bleeding episodes in previously treated children (0-12 years) with hemophilia B. The trial contained a main phase of 52 weeks, followed by an extension phase (currently ongoing).

In both the main phase and the extension phase, 40 IU/kg N9-GP was administered once weekly for prophylaxis in all children. Since weight-adjusted clearance of rFIX is approximately 1.7 times higher in children than in adolescents and adults,[57] the 10 IU/kg once-weekly dose was not used in the pediatric trial.

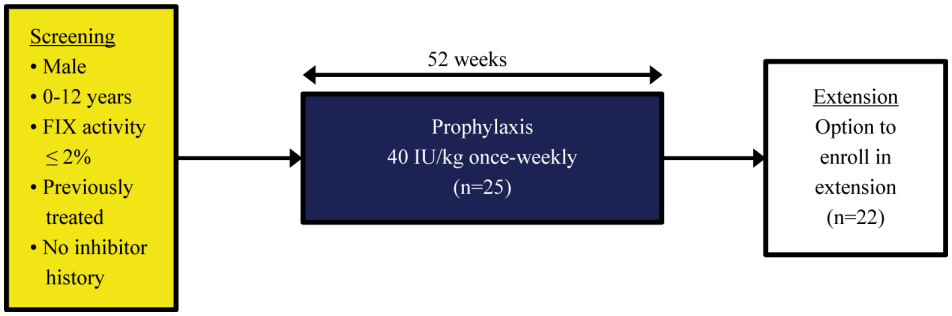
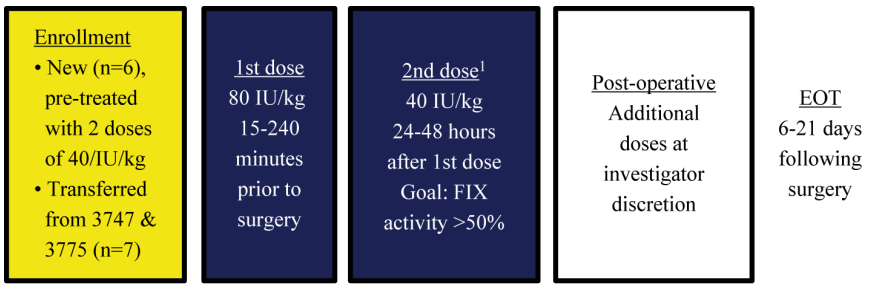


Figure 6–4 Overview of the pediatric Trial 3774

6.1.3.4 Major surgery in adolescents and adults (Trial 3773)

This was an open-label trial evaluating efficacy and safety of N9-GP in major surgical procedures in patients with hemophilia B. Patients could be recruited from Trials 3747 and 3775, and new patients not previously exposed to N9-GP could also be included into the trial (Figure 6–1). After completion of Trial 3773, patients were offered to continue on prophylaxis or on-demand treatment in Trial 3775. The trial design is presented in Figure 6–5.



1) Second dose was recommended in protocol

Figure 6–5 Overview of the major surgery Trial 3773

6.1.4 Enrolment criteria

In all trials, patients with severe and moderately-severe hemophilia B and a FIX activity level ≤2% and no history of FIX inhibitors were included.

For Trials 3747, 3774, 3775, eligible adolescents and adults were aged 13-70 years with a documented history of at least 150 exposure days to any FIX product.

For Trial 3774, eligible children were aged 0-12 with a documented history of at least 50 exposure days to FIX products.

For Trial 3895, eligible children were aged <6 years with no more than 3 exposure days to FIX products.

6.1.5 Baseline patient characteristics

The clinical trial program included patients from most major regions of the world. Demographic and baseline information did not differ between regions and the results of the global study population should be considered applicable globally and therefore appropriate for US application. A total of 32 patients (27.8%) in the clinical program were from the US and based on the above mentioned similarities across regions, the information is considered applicable to the U.S. population and to U.S. medical practice.

6.1.6 Endpoints and statistical methods

6.1.6.1 Primary objectives

The primary objective of Trials 3747, 3775 and 3774 was to investigate the immunogenicity of N9-GP. In the major surgery study (Trial 3773), the primary objective was to evaluate the hemostatic effect of N9-GP in patients undergoing major surgery.

6.1.6.2 Primary endpoints (except surgery trial 3773)

The primary endpoint in Trials 3747, 3775 and 3774 was the incidence of FIX inhibitors. A FIX inhibitor was defined as a titre ≥0.6 BU on two consecutive tests.

6.1.6.3 Secondary confirmatory efficacy endpoints

Trial 3747 was the only trial that had confirmatory secondary endpoints. In this trial, two confirmatory secondary endpoints were defined:

- **Hemostatic efficacy**: Hemostatic effect of N9-GP when used for treatment of bleeds was evaluated according to a predefined four-point scale (excellent, good, moderate or poor) by counting excellent and good as success while moderate and poor as failure (Table 6–2). The success rate (excellent or good) was estimated using a logistic regression model with treatment arm as fixed effect, where repeated measurements within the same patient was accounted for by assuming exchangeable correlation matrix.
- **Annualized bleeding rate (ABR)**: Annualized bleeding rates including during routine prophylaxis were estimated using a Poisson regression model including dose as a factor, allowing for over-dispersion and using treatment duration as an offset. Estimated annualized bleeding rates were presented with 95% confidence intervals (95% CI).

Table 6–2 Hemostatic efficacy in treatment of bleeds (Trials 3747, 3775, 3774)

	Definition	Treatment	Outcome
Excellent	Abrupt pain relief and/or clear improvement in objective signs of bleeding	Single infusion within 8 hours	Success
Good	Noticeable pain relief and/or improvement in signs of bleeding		
Moderate	Probable or slight beneficial effect after the first infusion	Requiring more than one infusion within 8 hours	Failure
Poor	No improvement, or worsening of symptoms	Within 8 hours after two infusions	

The two secondary confirmatory efficacy endpoints were pre-defined in the statistical analysis plan and were tested hierarchically to protect against false positive significances.

The first secondary confirmatory efficacy endpoint was hemostatic effect of N9-GP when used for treatment of bleeds, assessed as success/failure. This endpoint was to be tested as a confirmatory secondary endpoint only if the analysis of the primary endpoint (incidence of inhibitors) was successful. Non-inferiority (to 80%) would be concluded if the lower 95% confidence limit for the success rate was above 65%.

The second confirmatory efficacy endpoint was the number of bleeds during routine prophylaxis. This endpoint was to be tested as a confirmatory secondary endpoint only if the analysis of the primary endpoint and the first confirmatory secondary endpoint were successful. In this test, the prophylactic effect of N9-GP was assessed by testing whether the upper confidence limit for the annualised bleeding rate in the 40 IU/kg once-weekly prophylaxis was significantly below 4.8 bleeds/patient year (corresponding to 60% reduction of a pre-defined annualized bleeding rate of 12 bleeds/patient year for on-demand patients). Once this was demonstrated, a similar confirmatory test would be performed for the 10 IU/kg once weekly prophylaxis.

The dosing recommendations were determined based on the following criteria:

- If 10 IU/kg gives adequate prophylactic protection (defined as bleeding rates significantly below 4.8), and 40 IU/kg is not clearly better than 10 IU/kg, then the dosing recommendation should be 10 IU/kg.
- If 10 IU/kg gives adequate prophylactic protection, but 40 IU/kg is clearly better than 10 IU/kg, then the recommended dose should be 40 IU/kg.
- If 10 IU/kg does not give adequate protection but 40 IU/kg does, then the dosing recommendation should be 40 IU/kg.

In addition, the two doses would be compared statistically by a test with the null hypothesis that there is no difference between doses. A *post-hoc* analysis was performed comparing the estimated

annualized bleeding rates between the 10 IU/kg and 40 IU/kg prophylaxis arms where prior treatment was taken into consideration and with adjustment for historical bleeding rate. Furthermore, the cumulative hazard of bleed versus time since last administered dose was estimated in a Cox-proportional hazard model with treatment included as a fixed effect and patient modelled as a shared frailty. In this shared frailty model, the within-patient correlation is taken into account by assuming patient frailty follows a log-normal distribution.

No confirmatory statistical test of efficacy endpoints was planned for the three other trials (Trials 3773, 3774 and 3775) or the secondary endpoints that were not considered confirmatory in Trial 3747 as described above.

6.1.6.4 Secondary efficacy endpoints

Adolescents and adults pivotal trial (Trial 3747) – Secondary endpoints

- Number of infusions of N9-GP required per bleed; consumption of N9-GP; pre-dose (trough) FIX activity at steady-state (a mixed effect model on log-transformed plasma FIX activity with dose as a fixed effect and patient as a random effect was used; the estimated mean FIX trough level was presented using estimates back-transformed to the natural scale together with the 95% CI); patient reported outcomes (since patients responded to different instruments depending on their age at baseline, analysis sets specific to the age of the patients were created for the analyses).

Pediatric Pivotal (Trial 3774) – Secondary endpoints

- Hemostatic effect of N9-GP when used for treatment of bleeds (evaluated according to a predefined four-point scale, see Table 6–2); number of infusions of N9-GP required per bleed; consumption of N9-GP; estimated annualized bleeding rate during prophylaxis (annualized bleeding rates were estimated using a Poisson regression model with age-group as a factor, allowing for over-dispersion and using treatment duration as an offset; estimated annualized bleeding rates were presented with 95% CI); FIX trough activity at steady-state (see Section 5.1.1); patient reported outcomes (since patients responded to different instruments depending on their age at baseline, analysis sets specific to the age of the patients were created for the analyses).

Adolescent/Adult Extension (Trial 3775) – Secondary endpoints

- Hemostatic effect of N9-GP when used for treatment of bleeds (evaluated according to a predefined four-point scale, see Table 6–2); number of infusions of N9-GP required per bleed; consumption of N9-GP; estimated annualized bleeding rate during prophylaxis using a Poisson regression model (see Section 6.1.6.3); FIX trough activity (analysis complicated by switching between dosing groups); and patient reported outcomes (since patients responded to different instruments depending on their age at baseline, analysis sets specific to the age of the patients were created for the analyses).

6.1.6.5 Surgery endpoints

Major surgery (Trial 3773) – primary and secondary endpoints

- **Primary endpoint**: The primary endpoint in Trial 3773 was the hemostatic effect during surgery evaluated according to a predefined four-point scale (Table 6–3).

Table 6–3 Hemostatic efficacy in treatment of bleeds during surgery (Trial 3773)

Rating	Definition
Excellent	Better than expected/predicted in this type of procedure
Good	As expected in this type of procedure
Moderate	Less than optimal for the type of procedure but hemostatic response maintained without change of treatment regimen
Poor	Bleeding due to inadequate therapeutic response with adequate dosing, change of regimen required

- **Secondary endpoints:** Secondary assessments of intraoperative hemostasis included utilization of N9-GP (IU/kg) during surgery, transfusion fulfilling transfusion criteria during surgery, hemoglobin pre- and post-surgery (0, 1, and 24 hours). Hemostatic efficacy in the post-operative period through day 6, and from days 7-13 if still hospitalized, was evaluated based upon utilization of N9-GP (total consumption in IU/kg), transfusion requirements fulfilling transfusion criteria, hemoglobin, drainage volume, and wound hematoma.

In Trial 3773, major surgeries were defined as any invasive operative procedure that required several days of FIX substitution therapy and/or where any one or more of the following occurred: a body cavity was entered; a mesenchymal barrier (e.g. pleura, peritoneum or dura) was crossed; a fascial plane was opened; an organ was removed or normal anatomy was operatively altered. All patients undergoing major surgery received a single infusion of 80 IU/kg of N9-GP no more than 4 hours prior to the planned procedure. Postoperatively, the patients received fixed doses of 40 IU/kg, repeated at the investigator's discretion. The protocol recommended 2 doses of 40 IU/kg within the first 6 postoperative days.

Minor surgery (Trials 3747, 3775, 3774)

Minor surgery (defined as any invasive operative procedure where only the skin, the mucous membranes or superficial connective tissue were manipulated) was allowed within Trials 3747, 3775, and 3774. A dose of 40 IU/kg N9-GP prior to minor surgery was recommended to prevent perioperative bleeding.

No specific evaluation was planned around minor surgery. The number of doses utilized around minor surgery was captured.

6.2 Efficacy results

6.2.1 Study population

Of the total 115 previously treated patients who were exposed to N9-GP, 105 were included in one or more of the four phase 3 trials that contribute with efficacy data (Trials 3747, 3773, 3775 and 3774) and these patients make up the total patient population for efficacy assessments. The trials were global and included patients from Canada, France, Germany, Italy, Japan, Macedonia, Malaysia, Netherlands, Romania, Russian Federation, South Africa, Taiwan, Thailand, Turkey, United Kingdom and the United States of America. The majority of the patients (61.9%) were White and of non-Hispanic/non-Latino ethnicity (95.2%).

Of the 105 patients, 64 (61%) patients were on prophylaxis prior to the trial, and 41 patients (39%) received on-demand treatment prior to the trial. For patients on prophylaxis prior to trial entry, around 70% had used rFIX products, while the remaining had used pdFIX products. For patients receiving on-demand treatment prior to trial entry, around 44% had used rFIX products, while the remaining had used pdFIX products.

The population in the pediatric trial (Trial 3774) consisted of 25 male patients with severe hemophilia B. Twelve (12) patients were in the age group of 0–6 years (mean age 3.1 years) and 13 patients were in the 7–12 years age group (mean age 9.6 years).

6.2.2 Primary endpoint (inhibitor formation)

The primary endpoint for the trials in previously treated patients (Trial 3747, 3775, 3774 main phase) was a safety endpoint, formation of anti-FIX antibodies (inhibitors). There were no inhibitors reported in 105 previously treated patients in the 3 trials.

6.2.3 Treatment of bleeds

Fixed doses of 40 IU/kg (mild/moderate bleeds) or 80 IU/kg (severe bleeds) were recommended for treatment of bleeds. Only one severe bleeding episode was reported. The recommended dose was used where no dose adjustment was done in the clinical trials.

6.2.3.1 Pooled analysis of bleeds (Trial 3747, 3775, 3774)

Hemostatic effect: The hemostatic effect of N9-GP when used for treatment of bleeds was evaluated according to a predefined four-point scale of excellent, good, moderate or poor. Excellent and good hemostatic responses were considered as successful treatments, while moderate and poor hemostatic responses were considered as treatment failures. A total of 79 out of 105 (75%) patients experienced 597 bleeds of which a hemostatic response was reported for 591 bleeds and not reported for the remaining 6 bleeds. The overall observed success rate for the 591 bleeds was 93.2%. For the pooled results of all trials, the success rates for patients on prophylaxis (92.5%) and on-demand (94.4%) and across age groups were comparable.

Number of infusions to treat a bleed: Of the total 597 bleeds reported from all trials, 521 bleeds (87.3%) were controlled with 1 infusion, 60 bleeds (10.1%) were controlled with 2 infusions, 8 bleeds (1.3%) were controlled with 3 infusions, and 8 bleeds (1.3%) were controlled with ≥4 infusions of N9-GP.

N9-GP dose used for treatment of bleeds: All but one of the total 597 bleeds reported across all trials were mild or moderate in severity. The mean total dose of N9-GP per bleed across all trials was 52.3 IU/kg. This is higher than the 40 IU/kg prescribed initial dose in the protocols for treatment of mild/moderate bleeds due to 76 bleeds (12.7%) requiring more than one infusion for control.

In the pivotal trial (Trial 3747), patients were treated with on demand only (n=15) or randomized to two prophylaxis regimens. Occurrence and treatment of bleeding episodes by treatment arm is shown in Table 6–4).

Table 6–4 Hemostatic effect in treatment of bleeds (Trial 3747)

	N9-GP Prophylaxis		On Demand	Total[a]
	40 IU/kg Once-weekly	10 IU/kg Once-weekly		
Summary of patients and bleeds				
Number of patients	29	30	15	74
Number of patients with bleeds, N (%)	16 (55%)	25 (83%)	14 (93%)	55 (74%)
Number of bleeds	70	132	143	345
Hemostatic response				
Success rate (% Excellent/Good)	97.1%	86.9%	95.1%	92.4%[b]
Percentage treated with 1 dose (%)	98.6%	84.1%	83.9%	87.0%
Total Dose per Episode (mean IU/kg)	46.2 IU/kg	57.5 IU/kg	41.9 IU/kg	42.3 IU/kg

[a]The prespecified first confirmatory endpoint was overall hemostatic efficacy in all bleeding episodes.
[b] The estimated success rate was 92.2% and the 95% CI (87.0 ; 95.6) were above the prespecified lower acceptable value of 65%. Analysis considering missing bleeding events as failures results in success of 91.3% (95% CI 85.9; 94.8).

6.2.3.2 Bleeding episodes in children aged 0-12 years (Trial 3774)

In the completed main phase of the pediatric trial (Trial 3774), a total of 42 bleeds occurred over a mean treatment period of 1.17 years. There were 5 of 12 children aged 0-6 with 11 bleeding episodes (0-3 per patient) and 10 of 13 children aged 7-12 with 31 bleeding episodes (0-8 per patient). Of these, only 12 (29%) were spontaneous bleeds. One patient accounted for 50% of the bleeds observed (6 of the 12 spontaneous bleeds).

Overall hemostatic response was 92.9% with 85.7% of bleeding episodes treated with a single infusion of N9-GP 40 IU/kg (Table 6–5). While older children (ages 7-12) had a greater range in number of bleeds, hemostatic efficacy was similar for younger and older children (Table 6–5).

Table 6–5 Hemostatic response by age groups – Trial 3774

	N9-GP 40 IU/kg Once-Weekly Prophylaxis		
	0 - 6 years	7 - 12 years	Total
Summary of patients and bleeds			
Number of patients	12	13	25
Number of patients with bleeds, N (%)	5 (41.7)	10 (76.9)	15 (60.0)
Number of bleeds	11	31	42
Bleeds per patients (min, max)	0, 3	0, 8	0, 8
Hemostatic response			
Success rate (% Excellent/Good)	90.9%	93.5%	92.9%
Percentage treated with 1 dose (%)	81.8%	87.1%	85.7%
Total Dose per Episode (mean IU/kg)	42.8 IU/kg	43.1 IU/kg	43.0 IU/kg

6.2.4 Prevention of bleeds (routine prophylaxis)

6.2.4.1 Pivotal trial prophylaxis results in adolescents and adults (Trial 3747)

Annualized bleeding rate

In Trial 3747 both 40 IU/kg and 10 IU/kg once-weekly prophylaxis were evaluated (Table 6–6).The statistical analysis was done hierarchically by testing if ABR in 40 IU/kg once-weekly was significantly below 4.8 (corresponding to 60% reduction of the pre-defined annualized bleeding episodes of 12 bleeds/patient year for on-demand patients) before performing a similar test on 10 IU/kg. The 40 IU/kg once-weekly prophylaxis met the pre-specified statistical test but the 10 IU/kg once-weekly prophylaxis did not.

Table 6–6 Annualized bleeding rates – all bleeds and spontaneous bleeds in Trial 3747

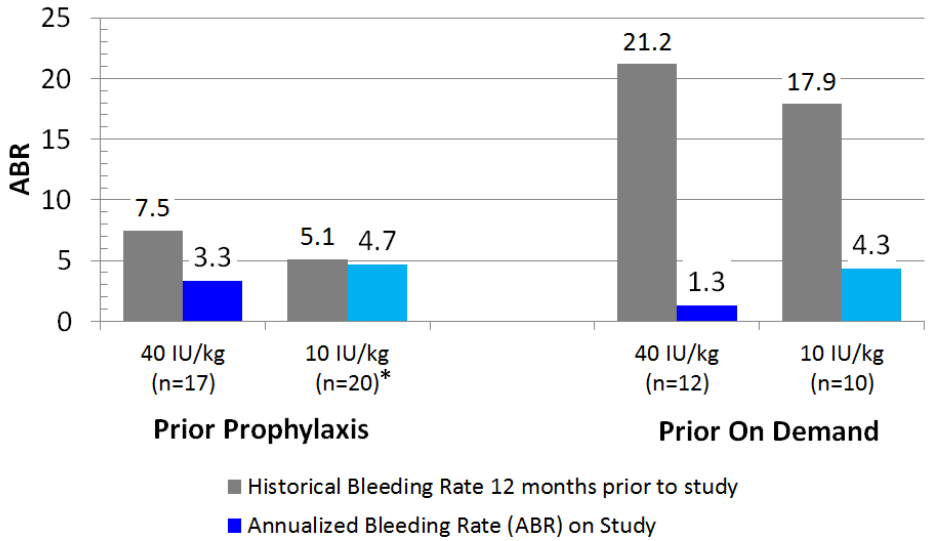
	N9-GP Prophylaxis Regimen	
	40 IU/kg once-weekly (N=29)	**10 IU/kg once-weekly (N=30)**
All bleeds		
Number of bleeds	70	132
Median ABR[a]	1.04	2.93
Interquartile range	0.00 ; 4.00	0.99 ; 6.02
Poisson estimate of ABR (95% CI)[b]	2.51 (1.42 ; 4.43)	4.56 (3.01 ; 6.90)
P-value[c]	0.013	0.402
Comparison of estimated ABR (adjusted analysis)		
40 IU/kg vs. 10 IU/kg (95% CI)	0.49 (0.05 ; 0.73)	
P-value[d]	0.033	
Spontaneous bleeds		
Number of bleeds	34	91
Median ABR	0.00	0.97
Interquartile range	0.00 ; 0.98	0.00 ; 4.01
Poisson estimate of ABR (95% CI)	1.22 (0.48 ; 3.10)	3.14 (1.78 ; 5.56)
Estimated ABR difference (adjusted)		
40 IU/kg vs. 10 IU/kg (95% CI)	0.65 (0.09 ; 0.87)	
P-value[d]	0.031	

[a] Annualized bleeding rate (ABR) is the mean number of bleeding episodes in a patient per year

[b] ABR estimates were based on Poisson regression model with dose as factor allowing for over-dispersion and using treatment duration as offset, adjusted for patient's prior treatment and historical ABR. Difference = 1 - ABR relative risk.

[c] 1-sided test of the null hypothesis that the ABR is at least 4.8 evaluated at the 2.5% level.

[d] 2-sided test of the null hypothesis that there is no difference between the two doses evaluated at the 5% level (post-hoc analysis where prior treatment was considered and adjustment for historical bleeding rate was performed).



*Missing historical bleed rates for 2 patients

Figure 6–6 Historical and on trial ABR by pre-trial treatment and on trial prophylaxis regimen (Trial 3747)

More than half (n=39, 63%) of the adolescent and adult patients randomized to the prophylaxis arms were on standard prophylaxis with recombinant or plasma-derived FIX before enrolment, including 13 patients with 20 target joints on pre-study prophylaxis. While 10 IU/kg once-weekly offered a prophylactic effect comparable to currently available treatments, 40 IU/kg once-weekly offered substantially better protection against bleeds (Figure 6–6).

The estimated annualized bleeding rate in the 40 IU/kg arm (2.51 bleeds/patient year) was 49% (p=0.033) lower compared to the 10 IU/kg arm (4.56 bleeds/patient year) (Table 6–6) when taking the prior treatment into account and adjusting for their historical bleeding rate. The difference was also consistent for spontaneous bleeds which was 65% lower (p=0.031) (Table 6–6). Patients treated with 10 IU/kg were 1.9 times more likely to have a bleed (P=0.012) and 2.7 times more likely to have a spontaneous bleed (p=0.014) than patients treated with 40 IU/kg.

Resolution of target joints

Arthropathy caused by recurrent bleedings into a joint can result in irreversible damage and is a major cause of morbidity in patients with hemophilia. Usually, a target joint is defined by ≥3 spontaneous bleeding episodes within 6 months. When there have been ≤ 2 bleeding episodes into the joint within 12 consecutive months the joint is no longer considered a target joint.

Resolution of target joints predicts for long-term, clinically meaningful minimization of progression of musculoskeletal damage. Prophylaxis with 40 IU/kg once-weekly demonstrated a dramatic improvement in number of target joints which was not observed with lower FIX levels in the

10 IU/kg dose regimen; 67% and 8% of patients in the 40 IU/kg and 10 IU/kg arm, respectively, experienced no bleeds in their target joint during the pivotal Trial 3747. The outcome was that 90% and 58% of the target joints in the 40 IU/kg and 10 IU/kg arm, respectively, were no longer considered target joints at the end of the trial, which was a statistically significant difference between the two arms (p=0.031) (Table 6–7).

Table 6–7 Target joints – Trial 3747

	N9-GP Prophylaxis	
	40 IU/kg Once-weekly	**10 IU/kg Once-weekly**
PedNET definition		
Number of patients with target joints at baseline	15	13
Number of patients with no bleeds in target joints at end of trial	10 (67%)	1 (8 %)
Test: 40 IU/kg vs 10 IU/kg**	**p=0.002**	
ISTH definition		
Number of target joints at baseline	20	19
Number of target joint bleeds during trial	19	49
Number of resolved target joints*at end of trial	18 (90%)	11 (58%)
Test: 40 IU/kg vs 10 IU/kg**	**p=0.031**	

According to the recent ISTH definition, a target joint is defined by ≥3 spontaneous bleeding episodes within 6 months. When there have been ≤ 2 bleeding episodes into the joint within 12 consecutive months the joint is no longer considered a target joint.

* i.e., target joints with ≤ 2 bleeds during Trial 3747 (52 weeks).

** 2-sided Fisher's exact test of the null hypothesis that there is no difference between the two doses in Number of patients with no bleeds in target joints at end of trial (PedNET definition) or number of resolved target joints at end of trial (ISTH definition) evaluated at the 5% level.

Patients who enrolled in the pivotal trial (Trial 3747) receiving pre-study prophylaxis were split across the 40 IU/kg and 10 IU/kg prophylaxis arms. For the 6 patients randomized to N9-GP 40 IU/kg once-weekly prophylaxis with 9 target joints, 56% of the target joints did not bleed. In contrast, all of the 7 patients randomized to 10 IU/kg with 11 target joints experienced continued bleeding into the prior target joints in the trial. This demonstrates that prophylaxis based upon current treatment goals, reflected in the 10 IU/kg once-weekly group, is unable to resolve target joint bleeding, and further, the advantages of higher FIX doses achieved with the 40 IU/kg dose in resolution of target joints.

Health-related quality of life

The 40 IU/kg once-weekly prophylaxis regimen demonstrated improvement from baseline to end of trial for both EQ-5D Visual Analog Scale and Haem-A-QoL Overall Score and domains of feeling, sports, and partnership, and significant improvement compared to the 10 IU/kg once-weekly

regimen for Haem-A-QoL (p=0.049) (Table 6–8). These measures showed consistent improvement with the 40 IU/kg dose that was not attained with the 10 IU/kg dose and align with the other efficacy measures which support that patients achieving higher FIX levels gain better efficacy outcomes and corresponding higher health-related quality of life. For an overview of the quality of life measures used in the clinical development program, please see Appendix II in Section 12.

Table 6–8 Quality of life scales showing a beneficial effect of once-weekly prophylaxis with 40 IU/kg compared to 10 IU/kg – Trial 3747

	N9-GP Prophylaxis Regimen	
Quality of life scale	40 IU/kg Once-Weekly (N=29)	10 IU/kg Once-weekly (N=30)
Haem-A-QoL scorea		
Number of adults (aged ≥ 17), n	22	24
Mean change from baseline (SD)	-6.4 (8.5)	1.7 (11.5)
p-value (40 IU/kg vs. 10 IU/kg)b	0.049	
EQ-5D VAS scorec		
Number of patients (age ≥ 13), n	29	30
Mean change from baseline (SD)	8.2 (17.2)	-0.3 (25.5)
p-value (10 IU/kg vs. 40 IU/kg)b	0.514	

a For Haem-A-QoL, a positive change indicated a worsening while a negative change indicated an improvement.
b p-values from a non-parametric Mann-Whitney test.
c For EQ-5D VAS, a negative change indicated a worsening while a positive change indicated an improvement.

6.2.5 Prophylaxis results in children (Trial 3774)

Annualized bleeding rate

N9-GP 40 IU/kg once-weekly prophylaxis in children was effective in preventing bleeding. Overall, 10 of 25 (40%) children had no bleeding episodes. The estimated (median) annualized bleeding rate for children was 1.44 (1.0) (Table 6–9). Younger children (aged 0-6) had a lower bleeding rate (mean 0.83, median 0.00) than older children (aged 7-12, mean 1.96, median 2.00).

Table 6–9 Annualized bleeding rate on N9-GP Prophylaxis by age groups – Trial 3774

	N9-GP 40 IU/kg Once-Weekly Prophylaxis		
	0 - 6 years	7 - 12 years	Total
Summary of patients and bleeds			
Number of patients	12	13	25
Number of patients with bleeds, N (%)	5 (41.7)	10 (76.9)	15 (60.0)
Annualized bleeding rate			
Individual ABRs			
Mean (SD)	0.83 (1.13)	1.96 (1.88)	1.42 (1.64)
Median	0.00	2.00	1.00
Interquartile range	0.00 ; 1.78	0.68 ; 2.89	0.00 ; 2.06
Min ; Max	0.00 ; 3.00	0.00 ; 6.51	0.00 ; 6.51
Poisson estimate of ABR	0.87	1.88	1.44
95% CI	0.38 ; 2.01	1.14 ; 3.09	0.92 ; 2.26

Poisson estimate: Based on a Poisson model including age group as fixed effect, allowing for over-dispersion and using treatment duration as offset.

Target joints

Two children with one target joint each experienced one traumatic bleeding episode each during the ~1 year of the main phase of the study. Based upon ISTH criteria, both would be considered as resolved.

6.2.6 Efficacy in surgery

6.2.6.1 Perioperative management in major surgery

Efficacy of N9-GP during major surgery was investigated in Trial 3773. A total of 13 major surgeries were performed in 13 patients. The primary endpoint was the hemostatic effect of N9-GP when used for surgical procedures. This was evaluated by the investigator/surgeon according to a predefined four-point response scale of excellent, good, moderate or poor. Treatment outcomes rated as excellent or good were referred to as successes while moderate or poor outcomes were referred to as failures.

The hemostatic effect of N9-GP during surgery was confirmed with an intraoperative hemostatic success rate of 100% in the 13 major surgeries in the trial. All 13 patients received a pre-operative dose of 80 IU/kg N9-GP on the day of surgery, which was considered effective, based upon no patients requiring additional doses on the day of surgery. In the postoperative days 1-6, the median number of doses was 2 (range: 0 to 4) and in the postoperative days 7-13, the median number of doses was 1.5 (range: 0 to 3). No other FIX products than N9-GP was used during surgeries.

Three (3) additional major surgeries were performed in 2 patients in Trial 3775, after completion of Trial 3773. The intraoperative hemostatic effect was confirmed with a success rate of 100%.

6.2.6.2 Perioperative management in minor surgery

Minor surgery was allowed in Trials 3747, 3775 and 3774. A dose of 40 IU/kg N9-GP prior to minor surgery was recommended to prevent perioperative bleeding. Of 36 minor surgeries, 35 were performed in patients on N9-GP 40 IU/kg or 10 IU/kg prophylaxis (Table 6–10). In 25 of these 35 minor surgeries (71.4%), patients received a single pre-operative dose of N9-GP 40 IU/kg; in 10 (28.6%) instances the surgery was conducted after receiving N9-GP as part of their prophylactic dosing regimen (or in 1 instance, after treatment of a bleed). The first post-operative dose after a minor procedure was most often the next scheduled prophylaxis dose, including for 85.0% of those on 40 IU/kg once-weekly and 93.3% of those on 10 IU/kg once weekly.

As it was not mandatory to report the hemostatic effect after a minor surgery, the assessment of hemostatic effect was available for 18 minor surgeries, all of which were rated as successful.

Table 6–10 Minor surgeries in patients on prophylaxis (Trials 3747, 3775, 3774)

	Prophylaxis Regimen		
	40 IU/kg once-weekly	**10 IU/kg once-weekly[a]**	**Total**
Summary of patients and minor surgery			
Number of patients with minor surgery	13	6	18
Number of minor surgeries	20	15	35
Last Dose Prior to Surgery, n (%)[b]			
Prophylaxis regimen	3 (15.0)	6 (40.0)	9 (25.7)
Surgery	16 (80.0)	9 (60.0)	25 (71.4)
Bleed treatment	1 (5.0)	-	1 (2.9)
First Dose After Surgery, n (%)[b]			
Prophylaxis regimen	17 (85.0)	14 (93.3)	31 (88.6)
Surgery	1 (5.0)	-	1 (2.9)
Treatment of bleed	2 (10.0)	1 (6.7)	3 (8.6)

[a] Applicable only to trials including adolescents/adults (Trials 3747, 3775)
[b] Two patients had two surgeries on the same day (n=33 surgical days)

7 Clinical safety

Summary

- A total of 115 previously treated patients (90 adolescents/adults and 25 children) were exposed to N9-GP in the 5 completed clinical trials with a total of 8801 exposure days (defined as a day where at least one N9-GP dose was administered) during approximately 170 patient years in trials.
- No clinical safety concerns were identified with use of N9-GP and the overall safety profile is similar to what has been reported for other FIX products. The nature and frequency of the reported adverse events in the N9-GP clinical trials do not reveal any unexpected safety signals.
- One death was reported for a patient with known risk factors for metastatic stage IV hepatocellular carcinoma. The death was evaluated as unlikely related to N9-GP by the investigator and Novo Nordisk.
- A total of 12 serious adverse events were reported in the completed clinical trials. Except for one event of hypersensitivity, all events were evaluated by the investigator and Novo Nordisk as unlikely related to N9-GP.
- No FIX inhibitors in previously treated patients and no thromboembolic events were reported .
- One previously untreated patient in an ongoing trial experienced an anaphylactic reaction and was subsequently found positive for development of FIX inhibitors.
- Allergic-type hypersensitivity reactions, including anaphylaxis, were reported with N9-GP. Based on the known pharmacological class effects for FIX products, there is no indication of increased risk of inhibitor development or allergic-type hypersensitivity reactions with N9-GP compared to other FIX products.
- Potential infusion site reactions with N9-GP included infusion site pain, infusion site swelling (as a result of subcutaneous N9-GP administration), infusion site erythema and infusion site rash.

7.1 Methodology

7.1.1 Safety assessments

The following safety information was collected in the N9-GP clinical development program:

- Adverse events
- Safety laboratory assessments
- Physical examinations
- Vital signs
- ECG
- Infusion site tolerability.

7.1.2 Important safety considerations

In addition to the routine safety assessments described in Section 7.1.1, safety monitoring for potential FIX inhibitors, hypersensitivity reactions, thrombotic events, and infusion site reactions was also performed in the N9-GP clinical development program, as these pharmacological risks are specific for FIX replacement products.

FIX inhibitors
Inhibitor development is a serious complication of FIX replacement therapy. Approximately 1-3% of previously treated patients with hemophilia B develop FIX inhibitors following exposure to a FIX replacement product, and as much as 5-10% for previously unexposed patients with severe hemophilia B.[42] Most cases of inhibitor development occur in early childhood after relatively few exposures to a FIX product (i.e., in previously untreated patients). In patients who develop inhibitors, the condition will manifest itself as an insufficient clinical response to FIX replacement therapy (lack of efficacy from prophylaxis and treatment of bleeds).

In all N9-GP clinical trials, patients were examined for the development of FIX inhibitors at scheduled visits. The modified Nijmegen Bethesda assay was used for FIX inhibitor detection.[68] A patient was defined as positive for FIX inhibitors if two analyses within approximately 14 days were at or above 0.6 BU. The threshold of ≥0.6 BU for diagnosis of an inhibitor was established via consensus of EMA experts.[69]

Hypersensitivity reactions
Allergic-type hypersensitivity reactions, including anaphylaxis, have been reported for marketed FIX products. The risk of reactions is highest during the early phases of exposure (i.e., initial 10-20 administrations). Patients who have developed FIX inhibitors are at an increased risk of anaphylaxis. N9-GP was not anticipated to be more immunogenic than other FIX concentrates, but patients in the clinical development program were monitored for hypersensitivity/anaphylactic reactions. While anaphylactic type reactions and nephrotic syndrome have also been reported following immune tolerance induction,[44] a treatment that consists of repeated exposure to replacement factor over a period of time to overcome inhibitors, N9-GP was not evaluated for this therapeutic use and thus nephrotic syndrome was not assessed during the development program.

Thrombotic events
High purity plasma-derived FIX products and recombinant FIX products have been associated with a very low frequency of thrombotic complications[44], therefore monitoring for thrombotic events was performed in all N9-GP trials.

Infusion site reactions
Infusion site reactions are known to occur with infusion of FIX products; thus, patients treated with N9-GP were monitored for potential infusion site reactions.

Suspected transmission of an infectious agent via trial product
Potential cases of transmission of infectious disease or agent were unlikely with recombinant replacement products but were to be reported as a serious adverse event in all N9-GP trials.

7.1.3 Safety population and pooling of data

Completed trials: The safety evaluation was based on all data from patients exposed to N9-GP in the 5 completed clinical trials (Trials 3639, 3747, 3773, 3775 and 3774 [main phase]).

Ongoing trials: For the ongoing pediatric extension trial and previous untreated patient trial, cases of deaths, serious adverse events and medical events of special interest (defined as medication errors, suspected transmission of an infectious agent, inhibitor formation, thromboembolic event, allergic and anaphylactic reactions) as of 01 April 2016 are included.

Please refer to Section 6.1 for details on the N9-GP development program.

Pooling of data:
There were no apparent differences in the safety profile of N9-GP between age groups (i.e., 0-1 years, 2-6 years, 7-11 years, 12-17 years, and 18-65 years) or treatment regimen (i.e., 40 IU/kg or 10 IU/kg once-weekly prophylaxis, or on-demand treatment) in the completed clinical trials (Trials 3639, 3747, 3773, 3775 and 3774 [main phase]). Therefore, safety data from all completed trials have been pooled and are reported together.

7.2 Clinical safety results

7.2.1 N9-GP patient exposure

In the 115 patients, a total of 8801 exposure days (defined as a day where at least one N9-GP dose was administered) were accrued. For the completed trials, this corresponds to a total of 170 patient years of exposure, where the total time was calculated from all patients from their first dose day until end of trial.

When exposure is included up until 01 November 2016, there is a total of 226 patient years, where 62 patients have been treated for more than 2 years (Table 7–1). As of this date, 20 children were still actively participating in the pediatric extension trial and 8 of these 20 children have been treated for more than 4 years.

Table 7–1 Number of patients exposed to N9-GP by age and duration of treatment – from completed and ongoing trials in previously treated patients

Duration of exposure by age	0-6 years (N=12)	7-12 years (N=13)	13-17 years (N=18)	18-70 years (N=72)	Total (n=115)
0 - <12 months	1	2	1	20	**24**
12 - <24 months	0	0	4	25	**29**
24 - <36 months	1	1	13	27	**42**
36 - <48 months	8	4	0	0	**12**
>=48 months	2	6	0	0	**8**

Exposure data for the patients in the pediatric trial (0-12 years) is up until 01 November 2016.
Additional safety data from the ongoing extension phase of Trial 3774 (i.e., from cut-off date 01 April 2016 in the 120-day Safety Update to cut-off 01 November 2016 for this document) has not been evaluated by the FDA.

Furthermore, the trial in previously untreated patients is currently ongoing, where the maximum duration of exposure in patients has exceeded 2 years.

7.2.2 Adverse events

There were 8801 exposure days in the clinical development program and a total of 647 adverse events. Of these, there were 12 serious adverse events, where only one event was considered related to N9-GP. An overview of all adverse events from the completed trials is shown in Table 7–2.

Overall, Novo Nordisk considers the safety profile for N9-GP to be similar to what is reported for other FIX products.

Table 7–2 Overview of adverse events - Trials 3639, 3747, 3773, 3775 and 3774 (main phase) - safety analysis set

Number of patients	115	
Total time in trial (years)	169.9	
Total number of exposure days	8801	
	Number of patients (%)	Number of events [event rate]
All adverse events	98 (85.2)	647 [3.8]
Serious adverse events	11 (9.6)	12 [<0.1]
Adverse events by severity		
Mild	93 (80.9)	559 [3.3]
Moderate	43 (37.4)	74 [0.4]
Severe	10 (8.7)	14 [<0.1]
Adverse events by relationship[a]		
Probably or possibly related	23 (20.0)	37 [0.2]
Unlikely related	97 (84.3)	609 [3.6]
Adverse events leading to withdrawal	2 (1.7)	2 [<0.1]

a: Causality for adverse events was judged by the investigator. The causality for one adverse event (overdose for patient number (b) (6) in Trial 3639) was captured in the safety database only and is therefore not included in this table which is based on data in the clinical database. The event was judged to be unlikely related by the investigator and possibly related by Novo Nordisk.
[Event rate]: Number of adverse events per patient years of exposure
All adverse events in this table are treatment emergent. Adverse events reported as a symptom of another adverse event are not included.
All treatment arms are pooled.
An exposure day is a day where the patient received at least one dose of N9-GP.
For the pediatric trial, data are included through the end of main phase for the last patient (7 April 2014).

7.2.3 Common adverse events

In the completed trials, the most commonly reported adverse events were nasopharyngitis, upper respiratory tract infection, contusion and cough (See Appendix III in Section 13). The rate of adverse events was higher in children aged 0-12 years than in adolescents and adults aged ≥13 years. As expected, this difference was mainly driven by frequent adverse events concerning common childhood diseases and did not raise any safety concern.

In the completed trials, a total of 37 adverse events in 23 patients were evaluated by the investigator as possibly or probably related to trial product corresponding to a rate of 0.2 events/patient years of exposure.

7.2.4 Deaths and other serious adverse events

A total of 12 serious adverse events in 11 patients were reported during the approximately 170 patient years of exposure in the completed trials (see Appendix III in Section 13).

Deaths
One adult patient was diagnosed with metastatic stage IV hepatocellular carcinoma after 499 days in the trial program and died after 570 days in the trial program. The death was evaluated as unlikely related to N9-GP by the investigator and Novo Nordisk. The patient had a history of hepatitis B and C and hepatic cirrhosis and was as such at risk of developing hepatocellular carcinoma.

Other serious adverse events
Of the remaining 11 serious adverse events only one event was considered related to N9-GP by the investigator and Novo Nordisk. This was a case of hypersensitivity in a single dose phase 1 trial and the patient recovered shortly after treatment.

Ongoing trials
From 08 April 2014 (the day after the data cut-off date for all completed trials) until 01 April 2016 (the data cut-off date for the most recent safety update provided to the FDA), 9 additional serious adverse events were reported in 3 patients from the ongoing pediatric extension trial and the trial in previous untreated patients. Only one patient had SAEs related to N9-GP:

- This previously untreated patient had a genotype that confers high risk for inhibitor development. The patient had an anaphylactic reaction after the fourth exposure with N9-GP, and FIX inhibitors were confirmed by laboratory assessments. The patient was withdrawn from the trial and recovered from the anaphylactic reaction.

7.2.5 Other significant adverse events

FIX inhibitors
No patients in the completed trials developed FIX inhibitors.

As lack of drug effect may indicate development of FIX inhibitors, all reported adverse events were evaluated in relation to possible development of FIX inhibitors. No adverse events of 'lack of efficacy' were reported and thus no indication of inhibitor development in any reported adverse events in previously treated patients.

One previously untreated patient in the ongoing trial developed FIX inhibitors, and thus, development of FIX inhibitors is an identified risk with N9-GP treatment and considered an adverse reaction.

Allergic-type hypersensitivity reactions
A total of 5 events in 4 patients were judged to be related to N9-GP by the investigator. The 5 adverse events were hypersensitivity, pruritus, eosinophilia, infusion site rash, and wheezing.

Thrombotic events
No thromboembolic events were reported.

Infusion site reactions
Potential infusion site reactions with N9-GP were identified and included events reported as infusion site pain, infusion site swelling (as a result of subcutaneous N9-GP administration), infusion site erythema and infusion site rash. A total of 5 infusion site reactions were reported in 4 patients in the completed trials. Infusion site reactions are considered adverse reactions; please see Table 7–3.

Adverse events leading to withdrawal
Two previously treated patients (1.7%) were withdrawn due to adverse events in the completed trials. One was a fatal event (death) and the other was a hypersensitivity reaction. One previously untreated patient was withdrawn due to an anaphylactic reaction. These events are described in Section 7.2.4).

Suspected transmission of an infectious agent via trial product
There were no events of suspected transmission of an infectious disease or agent via trial product in any of the N9-GP clinical trials.

7.3 Other safety observations

No clinically relevant changes associated with exposure to trial product have been observed for parameters of hematology, biochemistry, coagulation-related parameters and urinalysis in any of the clinical trials with N9-GP. In addition, no safety concerns were identified from vital signs, physical examinations or ECGs.

A total of five patients had positive titers for anti-CHO HCP antibodies during the N9-GP development program. One of the patients was positive prior to dosing with N9-GP. At the end-of-trial visit, three patients had low-titer positive tests. Positive tests were not considered to be associated to any clinical signs or symptoms when evaluating FIX activity levels or adverse events.

Anti-N9-GP binding antibodies were identified in 3 patients, of which 2 were positive prior to exposure to N9-GP. None of the anti-N9-GP binding antibodies had any inhibitory effect, and patients were only transiently positive. No patients were positive at the end-of-trial visit. Overall, detection of antibodies did not correlate with any adverse events or reduced trough FIX activity levels. Accordingly, no heightened risk was identified in any of the patient populations.

7.4 Safety assessments related to PEG accumulation

There are no reported safety concerns with regards to PEG accumulation arising from post-marketing data from PEGylated products. To evaluate the potential clinical impact from accumulation of PEG after long-term treatment with N9-GP, Novo Nordisk assessed the renal, hepatic, and neurologic safety data collected in the clinical program for any potential signal of adverse events.

Renal assessment
No systematic changes from baseline to end of trial were seen for estimated creatinine clearance or for renal clearance (eGFR). The assessments of creatinine and renal clearance did not show any clinically relevant changes associated with exposure to N9-GP.

Hepatic assessment
A significant number of patients with hemophilia B were infected with hepatitis C via administration of pooled factor concentrates, cryoprecipitate or fresh frozen plasma in the 1970s and early 1980s. During the clinical trials, some of the hepatitis C positive patients had abnormal liver parameters, which is expected for this population; however, no trends in safety findings possibly related to N9-GP were identified. In particular, there were no systematic changes from baseline to end of trial for any liver function parameters.

Neurologic assessment
Baseline and subsequent neurologic exams were performed in all trial subjects according to local procedure. Neurologic assessments were conducted as part of the physical examinations, and included general evaluation of the central and peripheral nervous system, functional assessment of the musculoskeletal system, and general appearance of the patient. Adverse events were collected throughout the clinical trials and captured any clinically significant changes identified from the physical examinations. No unexpected safety concerns were identified in the clinical trials and there have been no indications of neurological adverse reactions, and therefore nothing that could be attributed to potential PEG accumulation. Furthermore, there were no adverse events indicative of delays in child developmental milestones.

Conclusion
There were no unexpected safety findings, including renal, hepatic, or neurologic, over more than 4 years of treatment with N9-GP in clinical studies.

7.5 Summary of safety profile

Overall, no unexpected safety issues were identified during the N9-GP clinical development program with N9-GP.

Adverse drug reactions

Based on assessment of all pooled safety data from the completed trials, Novo Nordisk evaluated the possible causal relationship between all adverse events and N9-GP. The evaluation was based on a medical judgment and included assessment of frequency of the adverse event, pharmacological plausibility, timely causal relation, and frequency of the adverse event in the general population. Based on this evaluation, the adverse reactions listed in Table 7–3 are considered expected in previously treated patients treated with N9-GP.

Table 7–3 Adverse drug reactions in previously treated patients

System organ class	Preferred term	Number of patients with reaction/ Percentage of patients with reaction[a)]/
Immune system disorders	Hypersensitivity	1 0.9%
Skin and subcutaneous tissue disorders	Pruritus[b)]	3 2.6%
General disorders and administration site conditions	Infusion site reactions[c)]	4 3.5%

a): Number of patients with reaction by total number of unique patients exposed in all clinical studies (115).
b): Pruritus includes pruritus and ear pruritus.
c): Infusion site reactions include infusion site pain, infusion site swelling, infusion site erythema and infusion site rash.

In addition to the adverse drug reactions listed above, an anaphylactic reaction in an ongoing trial in previously untreated patients has occurred in close temporal association with development of FIX inhibitor following treatment with N9-GP. These events of anaphylactic reaction and FIX inhibitor development are considered expected in previously untreated patients with N9-GP.

7.5.1 Safety assessments in ongoing trials

As of 01 April 2016, no additional deaths, anaphylaxis or inhibitor developments were reported in the ongoing trials.

8 Plan for continued risk management post-approval

Summary

- Patients treated with N9-GP will continue to be closely monitored via routine pharmacovigilance.
- Novo Nordisk will continue to collaborate with registries with the aim of collecting specific safety data for N9-GP to monitor for effects of potential PEG accumulation after long-term treatment.
- To support long-term post-marketing safety, Novo Nordisk proposes to conduct a PASS with collection of specific data on hepatic, renal, and neurological function.
- Novo Nordisk will continue to collect long-term safety data from ongoing trials.

8.1 Planned pharmacovigilance activities post-marketing

Long-term safety data for treatment with N9-GP will be collected from ongoing clinical trials and from post-marketing sources which includes data from existing national/regional registries. In addition, safety data will be collected in a Post Approval Safety Study (PASS).

Patients treated with N9-GP will be closely monitored for any safety signal. Safety monitoring will focus on the following important risks (as defined in the Risk Management Plan):

Important identified risks

- Allergic reactions/hypersensitivity reactions
- FIX inhibitors

Important potential risks

- Thrombotic events
- Nephrotic syndrome following Immune Tolerance Induction (ITI)
- Inadequate treatment due to assay overestimation of FIX activity
- Potential accumulation of PEG after long-term treatment (see Section 8.1.2 for details)

8.1.1 Laboratory tests

The PEG moiety attached to the activation peptide of N9-GP interferes with the majority of the commonly used aPTT reagents, causing either under- or overestimation of the FIX activity level in the one-stage clotting assay. Assays that give accurate recovery measures of N9-GP in patient plasma have been identified and validated, and these include the chromogenic kits ROX FACTOR IX and BIOPHEN Factor IX, and a one-stage clotting assay with the aPTT reagent STA®-Cephascreen®. When qualified and validated one-stage clotting or chromogenic assays are not available in local laboratories, the use of a central laboratory with validated and accurate methods for measuring N9-GP FIX activity is recommended.

8.1.2 Potential accumulation of PEG after long-term treatment

FDA has raised specific concerns about the potential accumulation of 40 kDa PEG and how to assess for the potential clinical impacts of accumulation of PEG from long-term treatment with N9-GP.

Novo Nordisk is collaborating with the hemophilia community to explore opportunities on how to acquire additional clinical information through post-approval data collection that capture both general follow-up (including, but not limited to, laboratory parameters allowing evaluation of kidney and liver function), and neurological examinations. Any impact on neurodevelopment milestones in children is expected to be captured through the periodic clinical evaluations that are routinely undertaken in hemophilia patients, including neurological evaluation and detailed functional assessment of musculoskeletal systems.

Novo Nordisk will continue to engage with organizations such as ATHN (US) and collaborate with national and international patient registries such as European registries (EUHASS and PEDNET) to collect long-term safety data for treatment with N9-GP in a prospective manner to confirm the established safety profile.

Further, the FVIII and FIX Scientific Subcommittee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH) is working to harmonize data collection of safety and efficacy endpoints of novel/long-acting products in post-approval surveillance. This project will involve representatives from existing registries (including EUHASS and PEDNET), patient associations, regulatory authorities and pharmaceutical manufacturers of the long-acting products. Novo Nordisk is committed to work with the ISTH-SSC on this project to secure monitoring of long-term safety and efficacy of novel long-acting products, including N9-GP.

8.1.3 Post approval safety study (PASS)

To further strengthen the collection of long-term post-marketing safety data, Novo Nordisk proposes to conduct a PASS with collection of specific data on hepatic, renal, and neurological function.

8.1.4 Continuous safety data collection from ongoing clinical trials

Novo Nordisk will continue to collect long-term safety data from ongoing trials in pediatric patients. As of 01 November 2016, 20 pediatric patients were still enrolled in the trial with 40 IU/kg once-weekly prophylaxis with N9-GP, where 8 of the 20 children have been treated for more than 4 years. All pediatric patients are continuously monitored for clinical and laboratory safety, including physical and neurologic examinations and assessment of hematology, renal and hepatic parameters. The planned duration of treatment of children in the ongoing extension trial is scheduled until N9-GP is commercially available in the respective countries. Hence, the total treatment duration of the trial is expected to be more than 6 years for some pediatric patients with the current expected schedule for regulatory approval in all countries.

Furthermore, the trial in previously untreated patients will continue until 40 patients have completed 100 exposure days (2 years of treatment). As of 01 November 2016, a total of 17 patients have been treated with N9-GP. All patients are monitored for clinical and laboratory safety throughout the trial.

8.1.5 Conclusion

Novo Nordisk plans to monitor the safety of N9-GP through registries, PASS, as well as ongoing clinical trials. Special focus will be on potential accumulation of PEG, with monitoring of hepatic, renal and neurological function.

9 Benefits and risks

Benefits

Higher FIX Activity: N9-GP provides higher FIX levels than those delivered by the current standard and extended half-life products with a convenient once-weekly dose. N9-GP 40 IU/kg once-weekly achieved non-hemophilia FIX levels for 80% of the week for adolescents/adults; on average, children were above 15% FIX activity for the entire study period, and older children were above 19%.

- In a head-to-head comparison in a Phase 1 trial, the half-life of N9-GP was approximately 5 times longer than the half-life of BeneFIX® which, for decades, was the only commercially available rFIX product.[70]
- In adolescents and adults, N9-GP 40 IU/kg once-weekly achieved higher FIX levels in clinical trials than pharmacokinetic modelling suggests could be achieved with standard rFIX dosed twice weekly and current extended half-life products dosed weekly.
- In younger children (0-12years), N9-GP 40 IU/kg once-weekly achieved in clinical trials higher FIX levels than pharmacokinetic modelling suggests could be achieved with standard rFIX dosed twice weekly or current extended half-life products dosed weekly.

Clinical Outcomes: The higher FIX levels observed during prophylaxis treatment with N9-GP 40 IU/kg once weekly results in successful bleed control, reduced annualized bleeding rates and spontaneous bleeding rates, resolution of target joints, enhanced quality of life, and prevention of bleeding during major and minor surgical procedures.

- **Bleed Control**: In all trials and age groups, N9-GP bleed treatment with the 40 IU/kg dose was effective in the treatment of bleeds. Overall, 93.2% of the bleeds were successfully treated with N9-GP, including 97% of bleeds in adolescents and adults and 93% of bleeds in children; the results are comparable to or higher than what has been reported for standard and other extended half-life rFIX products.[20-23]

- **Bleed Prevention**: 40 IU/kg N9-GP was effective in preventing bleeding, including spontaneous and joint bleeding, in all age groups with a simple once-weekly regimen. N9-GP 40 IU/kg prophylaxis resulted in low median annualized bleeding rates (adolescents and adults: 1.04; children: 1.0) and spontaneous bleeding rates (adolescents and adults: 0.0; children: 0.0). Of the 54 children, adolescents and adults on prophylaxis with 40 IU/kg once-weekly, 39 (72%) patients did not have a spontaneous bleed during the treatment period of approximately one year. Adolescents and adults on prophylaxis with 40 IU/kg had a lower ABR than those with 10 IU/kg (estimated mean 2.51 vs 4.56, p=0.033); patients treated with 10 IU/kg were 1.9 times more likely to have a bleed (P=0.012) and 2.7 times more likely to have a spontaneous bleed (p=0.014) than patients treated with 40 IU/kg.

- **Target Joint Resolution**: Once-weekly prophylaxis with 40 IU/kg N9-GP resulted in substantial improvement in target joints across all age groups. Patients receiving 40 IU/kg were more likely to have no bleeding in target joints (40 vs 10 IU/kg: 67% vs 8%). In addition, using the ISTH criteria for target joint resolution (≤2 bleeds into the joint within 12 consecutive months)[24], both adolescents and adults on 40 IU/kg were more likely to have target joints resolved (18/20 [90%]) than those treated with 10 IU/kg (11/19 [58%]). In children, two patients with two target joints both had resolution of their target joints. These results indicate that once-weekly prophylaxis with 40 IU/kg N9-GP has the potential to prevent repeated bleeding in severely damaged joints.

- **Quality of Life**: Prophylaxis with 40 IU/kg once-weekly positively impacted health-related quality of life (HRQoL), while 10 IU/kg once-weekly did not. Adult patients (age ≥17) receiving 40 IU/kg once-weekly reported significant improvements in the overall total score for the HAEM-A-QOL (p=0.017) and in EQ-5D VAS (p=0.030).

- **Major and Minor Surgery**: N9-GP provided effective perioperative management in 16 major surgeries and in 35 minor surgeries where efficacy was assessed. The hemostatic effect of N9-GP during surgery was confirmed with an intraoperative success rate of 100% in the 16 major surgeries performed, including 9 major orthopedic procedures in patients ages 15-56 with substantially fewer doses than current standard rFIX. A total of 36 minor surgeries were performed with N9-GP, of which 35 were performed in patients on prophylaxis. In 25 of the 35 minor surgeries (71.4%), patients received a single pre-operative dose of N9-GP 40 IU/kg; in 10 (28.6%) instances the surgery was conducted without an additional pre-operative dose.

- **Dose Adjustment and Monitoring:** There was no dose adjustment performed during any of the clinical trials. The high factor IX levels achieved with 40 IU/kg once-weekly prophylaxis in all age groups reduced the need for monitoring.

Risks

N9-GP has demonstrated a safety profile similar to that of other currently approved Factor IX therapies, with no inhibitors in previously treated patients, no thromboembolic events, and an expected rate of allergic reactions.

Non-clinical and clinical data support the safety of N9-GP. Furthermore, the long-term safety of PEG is supported by extensive post-marketing experience from 11 PEGylated protein therapeutics currently used in the US.

Non-Clinical: Non-clinical data have shown that PEG is eliminated and indicate that PEG steady-state concentrations will be reached in patients within 2 years in plasma and all tissues. No PEG-related vacuolation or histological changes were observed in tissues, including the choroid plexus,

in nonclinical studies where PEG exposure levels were approximately 7-fold higher than steady state clinical exposure.

Clinical: Based on the treatment of 115 patients, including >4 years of active and ongoing treatment in children, no unexpected safety concerns have been identified.

- **Exposure:** A total of 115 unique previously treated patients have been exposed to N9-GP in the completed clinical trials, with a total of 8801 exposure days, corresponding to a total of 170 patient years of exposure through 07 April 2014. When including the ongoing pediatric extension trial with 20 children actively treated for up to 4 years, this corresponds to a total of 226 patient years of exposure through 01 November 2016.

- **Inhibitors**: None of the previously treated patients developed FIX inhibitors. A previously untreated pediatric patient developed an inhibitor to FIX, which is not unexpected given the incidence of inhibitors in previously untreated patients with severe hemophilia B (5-10%).

- **Allergic/Hypersensitivity Reactions**: Allergic-type hypersensitivity reactions, including severe hypersensitivity, were reported in 4 previously treated patients (5 events). A previously untreated pediatric patient developed anaphylaxis. This is consistent with the occurrence with other FIX products.

- **Thromboembolic Events**: No thromboembolic events were reported with N9-GP.

- **Factor Assay:** Extended half-life products have been documented to interfere with specific one-stage clot factor assays, but generally not chromogenic factor assays.

- **PEG Accumulation:** Based upon preclinical data from N9-GP and other PEGylated molecules, the risk of potential adverse consequences of accumulation of PEG over prolonged treatment periods has been identified as a theoretical risk. The nonclinical data and clinical experience with N9-GP have not to date shown any adverse consequences. However, the Risk Management Plan includes collection of additional N9-GP long-term clinical safety and exposure data with enhanced detection of potential adverse reactions.

Conclusion

N9-GP is the only treatment option which can sustain higher FIX levels than current products with once-weekly dosing. The higher levels achieved with N9-GP 40 IU/kg once-weekly prophylaxis result in successful bleed control, reduced bleeding, resolution of target joints, enhanced quality of life, and successful performance of surgical procedures.

N9-GP has a clinical safety profile similar to that which is reported for other FIX products. Non-clinical and clinical trial data have not identified any specific adverse concerns related to PEG with N9-GP at the proposed clinical doses. Long term PEG safety is further supported by post-marketing

experience from other products for more than 20 years. The risk management plan includes measures to enhance the detection of potential adverse reactions.

N9-GP provides clinically meaningful benefits for control and prevention of bleeds, perioperative management, and routine prophylaxis which outweigh the uncertainty around the theoretical risk pertaining to chronic exposure to the PEG moiety.

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11 Appendix I - Nonclinical studies with 40 kDa PEG

Early in development, prior to introducing the Rowett nude rats for chronic toxicity for N9-GP, it was decided to characterize the 40 kDa PEG moiety alone. Therefore toxicity, distribution, excretion and metabolism studies were performed with the unconjugated 40 kDa PEG moiety, see Table 1.

Table 1 Overview of studies performed with 40 kDa PEG alone

Type of study	Details of study	PEG doses µg/kg/week	Fold to corresponding PEG dose with once weekly 40 IU/kg dose of N9-GP (~230 µg/kg)	Fold to corresponding HED PEG dose with once weekly 40 IU/kg dose of N9-GP (~230 µg/kg)[a]	Species
Distribution	Single dose, Quantitative Whole Body Radiography, radiolabelled PEG followed for 12 weeks	600 (single dose)	-	-	Wistar rat
Excretion and metabolism	Single dose, plasma, urine and feces collected for 12 weeks	1,000, 12,000, 100,000, 200,000 (single dose)	-	-	Wistar rat
Repeat dose toxicity	2 & 6 weeks	45,000 117,000	196 509	31 81	Wistar rat
	2 & 6 weeks 13 weeks	45,000 7,000	196 30	63 10	Cynomolgus monkey

[a]Conversion based on body surface area, human equivalent dose (HED), calculated by multiplying dose with 0.32 (monkey) and 0.16 (rat).[71]

PEG was shown to have a similar distribution pattern as N9-GP and other 40 kDa PEG proteins and to be excreted in urine (55.5-74.8%) and feces (12-13.4%).[72]

The repeat dose toxicity studies using these high PEG doses confirmed the findings reported for other 40 kDA PEGylated drugs,[14, 73-77] i.e. vacuole formation observed in macrophages (rat, 45,000 and 117,000 µg/kg/week PEG for 6 weeks) and in the choroid plexus epithelial cells (monkey, 45,000 µg/kg/week PEG for 6 weeks). No evidence of degeneration, inflammation or necrosis was associated with the presence of PEG in macrophages or choroid plexus. No PEG-related vacuolation was seen in monkeys dosed 7,000 µg/kg/week PEG for 13 weeks.

Vacuolation has been described as an adaptive response to accumulation of PEG in cells.[14]

12 Appendix II - Patient Reported Outcome Measures Used to Assess Quality of Life

EQ-5D (3 level)

Population: Adolescents and Adults

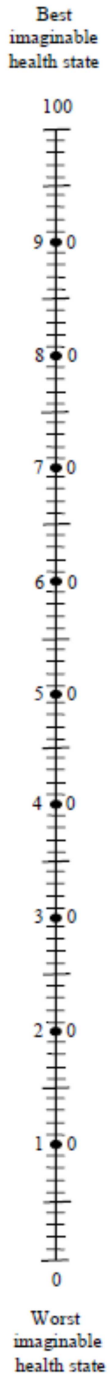
Overview: A standardized PRO developed for use as a simple, generic measure of health outcome ***TODAY*** for a wide range of conditions.

Outcome parameters:

- Simple descriptive profile on 5 dimension scores, version with 3 levels of response used
  - Mobility (no problems, moderate problems, unable)
  - Self-care (no problems, moderate problems, unable)
  - Usual activities (no problems, moderate problems, unable)
  - Pain/discomfort (none, moderate, extreme)
  - Anxiety/depression (none, moderate, extreme)
- **EQ5D Index** (Calculated index score based upon matching exact 5 item responses to an array of values, range -0.11 to 1.00)
- **Visual analog scale (VAS)** of ***YOUR HEALTH TODAY*** on a 10 cm standardized scale from 0 (worst possible health) to 100 (best possible health)

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.



HAEM-A-QOL

Population: Adults ≥ 17 years

Overview: The HAEM-A-QOL instrument is a widely used haemophilia-specific instrument designed to assess HRQoL in adults (aged 17+ years). Overall and domain scores range from 0 (best QoL) to 100 (worst QoL).

The 46 items organized into 10 domains; 6 domains ask the respondent to report on items from the perspective of their **experience in the past 4 weeks** (Physical health, feeling, view, sport, work, dealing) and 4 domains that ask the respondent to report on their **experiences recently** (Treatment, future, family planning, partnership). All items are rated on a Likert-type scale (1 "Never", 2 "Seldom", 3 "Sometimes", 4 "Often", and 5 "All the time").

Outcome parameters (scores/domains/items):

- Overall Score
- Physical health (5 items)
  - my swellings hurt
  - I had pain in my joints
  - it was painful for me to move
  - I had difficulty walking as far as I wanted to
  - I needed more time to prepare myself because of my condition
- **Feeling (4 items)**
  - my haemophilia was a burden for me
  - my haemophilia made me angry
  - I was worried because of my haemophilia
  - I felt excluded
- **View (5 items)**
  - I envied healthy people my age
  - I felt contented about my body
  - haemophilia made my life more difficult
  - I felt different from others because of my haemophilia
  - I was able not to think all the time about my haemophilia
- **Sport (5 items)**
  - I had to refrain from sports that I like because of my haemophilia
  - I had to refrain from sports like soccer
  - I did just as much sport as others
  - I didn't have the freedom to travel where I wanted
  - It was necessary for me to plan everything in advance
- **Work (4 items)**

  - I was able to go to work/school regularly in spite of my haemophilia
  - I was able to work/study like healthy colleagues
  - my everyday work/school activities were endangered by my haemophilia
  - I found it difficult to pay attention at work/school because I was in pain
- **Dealing (3 items)**
  - I tried to recognise early on when a bleed developed
  - I was able to tell whether or not I was bleeding
  - I was able to control my bleedings
- **Treatment (8 items)**
  - I was dependent on the factor concentrate because of my haemophilia
  - I was dependent on the physicians for the treatment of my haemophilia
  - I was annoyed about the amount of time spent having the infusions
  - I felt interrupted in my daily activities by the infusions
  - I was afraid of complications
  - I had problems with how my treatment was administered
  - I was afraid that in case of emergency other doctors don't know how to treat haemophilia
  - I was satisfied with the haemophilia centre
- **Future (5 items)**
  - I have been thinking that it will be difficult for me to lead a normal life
  - I have been expecting that things will get better in the future
  - I have been worrying that my condition is worsening
  - my life plans are influenced by my haemophilia
  - I am afraid that I will need a wheel chair
- **Family planning (4 items)**
  - I have problems having children
  - I am afraid that I cannot have children
  - I am afraid not to be able to take care of my children
  - I worry not to be able to raise a family
- **Partnership (3 items)**
  - I have been finding it difficult to date because of my haemophilia
  - I have been insecure in my relationships with women because of my haemophilia
  - I can't have a normal relationship because of my haemophilia

13 Appendix III – Safety tables

Table 1 Summary of most frequent adverse events reported by more than 5% of patients in the 5 completed clinical trials (Trials 3639, 3747, 3773, 3775 and 3774 [main phase])

	Total N(%) E[R]
Infections and infestations	
Nasopharyngitis	19(16.5) 35[0.2]
Upper respiratory tract infection	13(11.3) 20[0.1]
Influenza	11(9.6) 18[0.1]
Pharyngitis	7(6.1) 8[<0.1]
Gastroenteritis	6(5.2) 6[<0.1]
Injury, poisoning and procedural complications	
Contusion	15(13.0) 27[0.2]
Fall	9(7.8) 11[<0.1]
Head injury	6(5.2) 11[<0.1]
Skin abrasion	6(5.2) 11[<0.1]
Laceration	6(5.2) 9[<0.1]
Gastrointestinal disorders	
Vomiting	7(6.1) 14[<0.1]
Nausea[a]	7(6.1) 10[<0.1]
Respiratory, thoracic and mediastinal disorders	
Cough	15(13.0) 24[0.1]
Epistaxis	6(5.2) 10[<0.1]
Oropharyngeal pain	9(7.8) 10[<0.1]
Musculoskeletal and connective tissue disorders	
Pain in extremity[a]	11(9.6) 15[<0.1]
Arthralgia	10(8.7) 14[<0.1]
General disorders and administration site conditions	
Pyrexia	13(11.3) 24[0.1]
Fatigue[a]	8(7.0) 10[<0.1]
Nervous system disorders	
Headache[a]	12(10.4) 23[0.1]

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

Table 2 Listing of all serious adverse events from the 5 completed clinical trials (Trials 3639, 3747, 3773, 3775 and 3774 [main phase])

Patient number	Age (years)	Preferred term	ED	Relationship[a]	Severity	Outcome
Trial 3639						
(b) (6)	25	Hypersensitivity	1	Probable	Severe	Recovered
Trial 3747						
(b) (6)	45	Hip fracture	19	Unlikely	Severe	Recovered
(b) (6)	65	Skin ulcer	17	Unlikely	Severe	Not recovered
(b) (6)	15	Retroperitoneal haematoma	7	Unlikely	Moderate	Recovered
(b) (6)	14	Abdominal pain	4	Unlikely	Mild	Recovered
Trial 3775						
(b) (6)	41	Hepatocellular carcinoma	72	Unlikely	Severe	Fatal
(b) (6)	42	Road traffic accident	20	Unlikely	Mild	Recovered
(b) (6)	24	Faecaloma	78	Unlikely	Moderate	Recovered
(b) (6)	48	Post procedural infection	75	Unlikely	Moderate	Recovered
		Local swelling[b]	75	Unlikely	Moderate	Recovered
(b) (6)	33	Gastroenteritis	83	Unlikely	Severe	Recovered
(b) (6)	14	Femur fracture	68	Unlikely	Moderate	Recovered
Trial 3774						
(b) (6)	10	Food poisoning	17	Unlikely	Moderate	Recovered

ED: Exposure day
a: The relationship to trial product as judged by the investigator as either probable, possible or unlikely.
b: This serious adverse event was reported as a symptom of another serious adverse event (post procedural infection) and is not included in the summary statistics.
For Trial 3774, data are included through the end of main phase for the last patient (7 April 2014).
No serious adverse events were reported in Trial 3773.