FDA Briefing Document

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Blood Products Advisory Committee

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Coagulation Factor IX (Recombinant), GlycoPEGylated

N9-GP

Novo Nordisk, Inc.

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Disclaimer Statement

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Glossary

ABR	Annualized bleeding rate
AE	Adverse event
ADME	Absorption, distribution, metabolism, and excretion
aPTT	Activated partial thromboplastin time
AUC_{0-t}	Area under the curve
CA	Coagulant activity
CI	Confidence interval
CNS	Central nervous system
CSF	Cerebrospinal fluid
ECG	Electrocardiography
FIX	Coagulation factor IX
EM	Electron microscopy
EOT	End of treatment
IHC	Immunohistochemistry
IQR	Interquartile range
IV	Intravenous
IU	International unit
kDa	KiloDalton
KO	Knockout
N9-GP	Coagulation Factor IX (Recombinant), GlycoPEGylated
NHP	Nonhuman primate
NOAEL	No observed adverse effect level
PEG	Polyethylene glycol
PK	Pharmacokinetics
PTP	Previously Treated Patient
rFIX	Recombinant coagulation factor IX
$t_{1/2}$	Half-life
SD	Standard deviation
WBCT	Whole blood clotting time

1. Purpose of the Advisory Committee Meeting

Topic

Novo Nordisk is seeking FDA marketing approval for N9-GP, a Coagulation Factor IX (Recombinant), GlycoPEGylated, for use in adults and children with hemophilia B for control and prevention of bleeding episodes, perioperative management, and routine prophylaxis.

Issue

N9-GP (Coagulation Factor IX (Recombinant), GlycoPEGylated, N9-GP) is a recombinant FIX molecule. The addition of a 40 kiloDalton (kDa) polyethylene glycol (PEG) moiety to the FIX protein increases its half-life. N9-GP is therefore longer-acting and was developed for intravenous replacement therapy or prophylaxis on a less frequent basis. N9-GP is proposed for weekly administration and is anticipated to improve convenience of use for patients.

Novo Nordisk submitted this original BLA seeking approval for the following indications for adults and children: a) for control and prevention of bleeding episodes; b) for perioperative management; and c) routine prophylaxis. The evidence to support licensure for the proposed indications is from three Phase 3 trials and a Phase 3 extension study in adults and children. The effectiveness of this product is not a primary issue for consideration by this Advisory Committee. However, the nonclinical studies found PEG accumulation in the choroid plexus following repeat N9-GP dosing. The clinical trials did not find any safety signal that was clearly likely to be caused by PEG accumulation. However, it is unclear whether the clinical monitoring of neurologic function was adequate to detect all clinically important neurologic signs or symptoms.

FDA seeks the opinion of the Committee as to the safety of the product. Of interest to the FDA is the Committee's assessment regarding risks in the intended population, particularly in the pediatric and elderly populations, and particularly in the setting of chronic administration. FDA is also asking the Committee to consider whether monitoring, particularly of neurologic function, should be provided for the safety of patients or study subjects.

2. Overview of Hemophilia B

Hemophilia B is a blood clotting disorder caused by coagulation factor IX (FIX) deficiency or dysfunction. FIX is encoded by the F9 gene, which is found on the X chromosome. As a result, hemophilia B is almost exclusively found in males, although heterozygous females may exhibit a mild form of the disease. The incidence of hemophilia B is estimated to be approximately 1 case per 25,000-30,000 male births. Hemophilia B has a wide geographic distribution and is encountered in all racial and ethnic groups. Hemophilia B can be classified as severe (FIX coagulant activity (CA) < 1% of normal), moderate (CA 1% – 5%) or mild (CA 5% - 30%) [1].

The hallmark of hemophilia is hemorrhage into the joints (typically the ankles in children, and the ankles, knees, and elbows in adolescents and adults) resulting in permanent deformities, misalignment, loss of mobility, and extremities of unequal lengths. With mild hemophilia, hemorrhage is most likely to occur with trauma or surgery. The availability of virus-cleared, plasma-derived, and recombinant FIX

concentrates as effective treatment for bleeds and as prophylaxis has significantly improved outcomes in hemophilia B patients, who can now achieve a close to normal life span.

Treatment of hemophilia B requires regular infusions with FIX-containing preparations. FDA-approved products for the treatment of hemophilia B include recombinant FIX (BENEFIX, IXINITY, and RIXUBIS), plasma-derived FIX (MONONINE and ALPHANINE), or plasma-derived FIX complex (PROFILNINE and BEBULIN). Two long-acting recombinant FIX-fusion proteins, APROLIX (with Fc) and IDELVION (with albumin), are also available, which allow patients to infuse less frequently (every 7 days) in routine prophylaxis regimens.

The development of neutralizing anti-drug antibodies (often called "inhibitors" in coagulation literature) to FIX occurs in 3-5% of patients with hemophilia B. This is currently the most serious complication in the management of hemophilia B, and represents a major source of morbidity and mortality. The neutralizing anti-drug antibodies to a particular product often, though not always, cross-react with other FIX products as well as the patient's endogenous FIX.

3. Function of the Choroid Plexus

The choroid plexus (CP) is the tissue that actively secretes cerebrospinal fluid (CSF). There are four CPs found in the brain: two in the lateral ventricles, one in the third ventricle, and one in the fourth ventricle [2]. The ventricles are lined with the ependyma. The CPs are leaf-like structures which float in the CSF and are attached to the ependyma by a thin stalk. The CPs consist of epithelial cells that surround capillaries and connective tissue. It is these epithelial cells which form the blood-CSF barrier. The CP also holds various immune cells and is a gateway for immune cell entry into the CNS; these immune cells include macrophages, dendritic cells, and epiplexus cells (local antigen-presenting cells). Pericytes and macrophage-derived cells control entry of large molecules and maintain the blood-brain barrier [3, 4]. The CP is also responsible for maintaining the homeostasis of nutrients and osmotic load in the CSF [2, 5, 6].

4. Product Description

4.1. General Information about Coagulation Factor IX

The activated form of FIX is a vitamin K-dependent serine protease in the blood coagulation system, responsible for converting FX to its active form FXa. Factor IX is synthesized as a 461 amino acid precursor (primarily in the liver) and then secreted into plasma. FIX zymogen undergoes extensive co-translational and post-translational modifications, such as glycosylation (~17% carbohydrate by weight) and γ-carboxylation. It has a 46 amino acid N-terminal propeptide (which includes a 28 amino acid signal sequence) that is cleaved off during cellular processing of FIX. FIX circulates in plasma as a single-chain inactive zymogen of 415 amino acids. During blood clotting, the zymogen is activated by two distinct mechanisms: either by FXIa (intrinsic pathway) or FVIIa/tissue factor (extrinsic pathway). The activation of FIX results in the excision of the activation peptide (amino acid 145–180) that converts FIX into its active form (FIX$\alpha\beta$), where two polypeptide chains are linked together by a disulfide bond [7].

4.2. General Information about Novo Nordisk's N9-GP

Novo Nordisk's recombinant FIX (rFIX) is expressed in a Chinese hamster ovary cell line. The rFIX protein is purified using a manufacturing process that includes chromatographic and viral clearance steps. A 40-kilodalton (kDa) polyethylene glycol (PEG) moiety is enzymatically attached to the N-linked glycans on rFIX using (b) (4) as a linker. The product is further purified by (b) (4) to the Coagulation Factor IX (Recombinant), GlycoPEGylated product. The addition of the 40-kDa PEG moiety to rFIX is intended to increase its circulatory half-life. In this document, the product is referred to as N9-GP, but other equivalent names are GlycoPEGylated rFIX or GlycoPEG-rFIX, or GP-rFIX.

The molecular weight of N9-GP is approximately 98 kDa. The structure of N9-GP was determined and compared to licensed recombinant FIX products. The comparisons considered (b) (4)

A one-stage FIX clotting assay is used to determine the biological activity of N9-GP with a specific activity of about 152 IU/mg.

N9-GP is predominantly mono-PEGylated (b) (4)
. Additional PEGylated species that have been identified are: (b) (4)
. In addition, (b) (4) in early production batches), and other process- and product-related impurities (b) (4) were observed. The safety of these molecular species and impurities were assessed in non-clinical and clinical studies, and appropriate specifications have been established to control their levels in the commercial product.

PEGylated therapeutic products now range from PEG–protein to PEG–aptamer conjugates that are indicated for a variety of disorders, such as the treatment of Crohn's disease and stimulation of regrowth of white blood cells after chemotherapy [8]. The PEG moiety itself is also quite varied in structure, from linear chains to branched dendritic-like polymers, with molecular sizes ranging between 5 and 60 kDa. PEG is usually attached to the active ingredient of these products via reactions with amine-reactive groups. Comparison of the approved PEGylated products to N9-GP is challenging because of the heterogeneity of the products, their use in different disease states, and different dosing regimens. In addition, the approved similarly PEGylated products are expected to have shorter durations of exposure than the life-long treatment expected with prophylactic use of N9-GP.

5. Overview of the Nonclinical Program

The safety and activity of N9-GP were characterized in a nonclinical program that included in vivo efficacy testing, as well as *in vivo* pharmacokinetics, local tolerability, and single- and repeat-dose toxicity studies in FIX-deficient (hemophilic) mice and dogs, and in FIX-replete (i.e., wild-type) monkeys, rats, and rabbits. The nonclinical studies evaluated doses ranging from the proposed recommended prophylactic clinical dose of 40 IU/kg up to 3750 IU/kg.

Previous experience with similar recombinant and plasma-derived FIX products has demonstrated that the toxicities of exogenously administered FIX are extensions of its pharmacologic activity (i.e., hypercoagulability of blood, thrombosis, and thrombo-embolus formation in treated animals and patients). Additional expected nonclinical findings are development of neutralizing and non-neutralizing antibodies directed against the human FIX protein (i.e., immunogenicity), with the potential to cross-react and neutralize endogenous FIX in wild-type animals.

5.1. Pharmacology

The pharmacology of N9-GP was evaluated in proof-of-concept and secondary pharmacodynamics studies in murine and canine models of hemophilia B. These animals had a naturally occurring mutation/deletion of FIX function. Studies were also conducted in normal, FIX-replete (i.e., wild-type) nonhuman primates (NHPs).

Administration of N9-GP to hemophilic dogs at dose levels approximately equivalent to the human starting dose level restored the *ex vivo* whole blood clotting time (WBCT) activity and activated partial thromboplastin times (aPTT) to within normal limits, and the results were comparable to those obtained following dosing with the FDA-approved human FIX product. There were no effects of N9-GP or the approved rFIX product (BENEFIX®) on the hematology profiles in hemophilic dogs as compared to baseline, and no serious adverse effects or evidence of thrombogenicity were reported.

Administration of N9-GP in hemophilic mice at dose levels approximately equivalent to the human starting dose level (~6 to 250 IU/kg) restored blood loss (as measured by tail bleeding), *ex vivo* WBCT activity, and aPTT to within normal limits, and the results were comparable to those obtained following dosing with the approved human rFIX product. There were no effects of N9-GP, or the approved FIX product (BENEFIX®), on the hematology profiles in mice as compared to baseline levels. In addition, no serious adverse effects or evidence of thrombogenicity were reported.

In summary, animal pharmacology studies with N9-GP demonstrated the expected pharmacologic activity (i.e., pro-coagulant pharmacodynamics) in a murine and canine model of hemophilia B, and the results were similar to those obtained with FDA-approved human FIX products. There was no evidence of undesirable secondary pharmacologic activity (i.e., thrombogenesis) in FIX-replete monkeys dosed with PEG-FIX at dose levels up to 1300 IU/kg (32-fold greater than the equivalent human N9-GP starting dose). These data were used as proof-of-concept to support the rationale for administering N9-GP in clinical trials.

5.2. Pharmacokinetics

Pharmacokinetic studies with N9-GP were conducted concurrently with the pharmacology studies in the hemophilia B dogs and mice, and FIX activity was measured by both the one-stage clotting and chromogenic assays. With one-stage assays, the PK profiles from hemophilic dogs administered N9-GP showed dose-dependent increases in all parameters measured, and were comparable to those obtained when the dogs were dosed with an approved human recombinant FIX comparator. Similar results were obtained in FIX-replete monkeys administered N9-GP and an approved human FIX comparator product. Monkeys showed a dose-dependent increase in all pharmacokinetic endpoints. In hemophilia B mice, with both assays, the PK profiles from mice administered N9-GP showed dose-dependent increases in all parameters measured, and were comparable to those obtained when mice were dosed with the approved rFIX product (BENEFIX®). Similar results were obtained in FIX-replete monkeys and minipigs administered N9-GP and an approved human FIX comparator product.

After intravenous dosing in animal models, N9-GP displayed a terminal half-life ($t_{1/2}$), as measured by the chromogenic assay, ranging from 41 hours in the FIX-KO mouse and 76 hours in the minipig. Using the

one-stage clotting assay, $t_{1/2}$ ranged from 67 hours in the FIX-KO mouse and 113 hours in the hemophilia B dog. The $t_{1/2}$ of N9-GP was two- to six-fold longer compared to the approved human recombinant FIX comparator product in all animal models. Clearance was seven-fold lower in the minipig and 10- to 20-fold lower in hemophilia B mice and dogs when compared to BENEFIX®. Following acute intravenous administration, the *in vivo* plasma recovery (C_{max}) was 1.1-1.8 fold higher for N9-GP as compared to an approved human recombinant FIX comparator product.

Regarding the exposure ratios for the animal dose levels compared to the human dose level, the area under curve (AUC_{0-t}) was five-fold higher at the highest dose level in the rats (1200 IU/kg) and up to 75-fold higher at the highest dose level in the monkeys (3750 IU/kg) when compared to the proposed recommended prophylactic clinical dose of 40 IU/kg.

A series of PK studies in FIX-replete rats and monkeys showed that the N9-GP product tested in the nonclinical safety program was comparable to the product used in clinical trials, and that changes in manufacturing during the development program did not affect the critical PK profile.

5.3. ADME of N9-GP and 40-kDa PEG

Complete excretion of the 40-kDa PEG moiety was observed in a nonclinical study investigating the distribution and excretion of the radiolabeled 40-kDa PEG moiety in N9-GP after a single intravenous administration of a single high dose level (2.8 mg/kg equivalent to 420 IU mg/kg) in FIX-KO mice. PEG is predominantly excreted in urine and feces in a time- and dose-dependent manner.

Tissue distribution studies using quantitative whole body autoradiography were conducted in hemophilia B mice and FIX-replete rats administered a single dose of N9-GP or the 40-kDaPEG moiety alone. The results were similar in all studies for tracers and compounds for tissue distribution. Radioactivity was widely distributed, but mostly concentrated at the highest levels in highly-vascularized organs and tissues such as spleen, urinary bladder, blood, lung, bile ducts, periodontal membrane, kidney, adrenal, and liver; and to a much lesser extent in the brain and spinal cord.

The metabolism of N9-GP and the 40-kDa PEG moiety alone was evaluated in the urine, feces, and plasma samples from hemophilia B mice and FIX-replete rats. Results indicated that 40-kDa PEG circulates longer (up to 7.5-9 times longer) than N9-GP in plasma.

In summary, results indicate that 40-kDaPEG prolongs the circulation of N9-GP in plasma, is readily metabolized in the liver and kidney, and is excreted in the urine and feces. It also appears that there is some PEG accumulation in highly-vascularized organs and tissues. These data provide some insight regarding the metabolism and clearance of PEG from organs other than the choroid plexus; however, the metabolism of PEG may be different in humans compared to rodents.

The mechanism of clearance of PEG from the choroid plexus remains unknown.

5.4. N9-GP Toxicology

Six studies were conducted in FIX-replete rats (immune-competent and immune-deficient) and monkeys to evaluate the toxicity of IV-administered N9-GP. **Table 1** provides an overview of these toxicity studies.

Table 1. Overview of the six studies conducted to evaluate the toxicity of IV-administered N9-GP.

Study Number	Species	Dose Level(s) (IU/kg)	Dosing Regimen	Sacrifice Time Points
1	Wistar Rat	0 200 1000 2000	Single	24 hours post-administration
2	Cynomolgus Monkey	0 350 1300 3750	Repeat (once weekly for 4 weeks) *5-week recovery period**	4 weeks post-first administration (Terminal sacrifice) 5 weeks post-last administration (Recovery sacrifice)
3	Cynomolgus Monkey	200	Repeat (once weekly for 13 weeks) *5-week recovery period*	5 weeks post-last administration
4	Wistar Rat	25 200	Repeat (once weekly for 14 days)	14 days post-first administration
5	Rowett Nude Rat	0 40 1200	Repeat (twice weekly for 6 weeks) *2-week recovery period*	7-8 weeks post-first administration
6	Rowett Nude Rat	0 40 150 600 1200	Repeat (once weekly for 26 weeks) *26-week recovery period for a separate high-dose group only*	26 weeks post-first administration (Terminal sacrifice) 26 weeks post-last administration (Recovery sacrifice)

*high-dose animals had only one week of recovery

Study #1

Wistar FIX-replete rats dosed with a single intravenous injection of N9-GP at dose levels up to 2000 IU/kg (50-fold greater than the clinical starting dose of 40 IU/kg) demonstrated no systemic toxicities. All animals were sacrificed as planned following cessation of dosing. In addition, the histopathological findings were unremarkable and consistent with Wistar rats of that age. Per the test report, since no toxicity was observed and there was no decrease in survival, 2000 IU/kg of N9-GP was determined to be the NOAEL for acute dosing.

Study #2

A repeat-dose toxicity study with N9-GP was conducted in cynomolgus monkeys. Animals were administered N9-GP weekly for four weeks by bolus intravenous injection with dose levels equal to 350, 1300 and 3750 IU/kg. The reversibility of any signs noted during the dosing period was evaluated over a five-week recovery period (i.e., no test article administered). Statistically-significant differences and trends for N9-GP compared to vehicle control were reported for prothrombin time and aPTT. There was a dose-related extension of prothrombin times when compared to baseline samples. aPTT was reduced at 24 hours post-dose; however, this decrease is expected as it represents the pharmacological effect of N9-GP. There were no reported elevations of *ex vivo* biomarkers of thrombosis (i.e., thrombin, thrombin-anti-thrombin complex, D-dimer and prothrombin fragments 1+2 formation) at dose levels up to 1300 IU/kg/week. There was development of cross-reacting neutralizing antibodies in some animals, as expected.

Two animals in the 3750 IU/kg/week dose level group had to be euthanized at Day 28 and Day 36, respectively, prior to the scheduled sacrifice time point. One animal had significant sub-meningeal congestion/hemorrhage in the brain and acute inflammatory cell infiltration in the spinal cord. Both animals had hemorrhage associated with cellulitis in the skin/subcutis. Hemorrhage was determined to be the cause of demise for both animals. Both animals had cross-reacting neutralizing antibodies detected in their blood samples at the time of terminal sacrifice.

Three animals from the 3750 IU/kg/week dose level group were sacrificed early (Day 24), as scheduled. Microscopic findings in these animals indicated hemorrhage associated with cellulitis in the skin/subcutis. In addition, one animal had congestion/hemorrhage in the brain. These findings were similar to those observed in the animals from this group who were euthanized early on Days 28 and 36.

This study also included an evaluation of neurological/CNS and cardiovascular endpoints. Neurological/CNS and cardiovascular endpoints were evaluated on Days 0 and 2, and on Days 12, 16, 23, and 30 for the high-dose animals only (3750 IU/kg/week). Cardiovascular safety and toxicity endpoints such as blood pressure, heart rate, and ECG were evaluated during study. Basic neurological/CNS endpoints evaluated included autonomic, behavioral and neurologic assessments such as locomotor, alertness, reaction to stimuli, salivation, ptosis, piloerection, cyanosis, cutaneous blood flow, posture, balance/coordination, catalepsy, tremor, and convulsions. Although individual blood pressure measurements varied throughout the course of the study, there were no statistically significant trends. ECG results did not demonstrate a consistent pattern in the mean or individual data to indicate a negative effect on heart rate or ECG intervals and waveforms. Mild and transient body tremors were found in six of eight monkeys in the high-dose group. Tremors began hours to days post-dosing and abated within one hour. Tremors were not observed after the third weekly administration. The cause of the tremors was not clearly identified in the test report. There were no other significant neurological findings in any of the study animals.

Study #3
An additional repeat-dose toxicity study was conducted in cynomolgus monkeys to evaluate the immunogenicity of N9-GP. Animals were administered N9-GP by bolus intravenous injection once weekly for 13 weeks, followed by a five-week recovery period, at a dose level of 200 IU/kg. No systemic toxicity was observed; however, there was formation of neutralizing antibodies in all animals, as expected.

Study #4
A repeat-dose toxicity study comparing N9-GP to an approved recombinant human FIX (BENEFIX®) product was conducted in FIX-replete Wistar rats. Animals were administered N9-GP by bolus intravenous injection at dose levels of 25 or 200 IU/kg once weekly for 14 days. All animals remained healthy, with no early mortality or adverse clinical observations reported. There were some statistically-significant differences and trends for all groups administered N9-GP compared to the group administered BENEFIX® regarding mean clinical chemistry and aPTT. These differences, however, were not considered to be of toxicological significance.

Study #5
Additional nonclinical studies were conducted in Rowett nude rats to permit long-term toxicity studies without concern for development of cross-reacting neutralizing antibodies. A six-week, repeat-dose

toxicity study evaluating administration of N9-GP by bolus intravenous injection, followed by a two-week recovery period, at dose levels of 40 and 1200 IU/kg twice weekly was conducted. Trends for a reduction in aPTT were observed for some animals; however, these trends were transient. Two female animals administered 40 IU/kg were sacrificed early due to issues of the urogenital tract. However, these were not considered related to N9-GP since no such issues were seen in the remaining animals. With the exception of vacuolation in various organs (e.g., liver, adrenals, parotid salivary gland, stomach, lachrymal glands, choroid plexus, testes, kidneys), there were no other major histopathological findings in either control animals or animals administered N9-GP.

Study #6
The N9-GP was administered to Rowett nude rats by bolus intravenous injection at dose levels of 40, 150, 600 and 1200 IU/kg once weekly for 26 weeks. A separate group of animals received 1200 IU/kg once weekly for 26 weeks, followed by a 26-week recovery period. Animals exhibited a prolonged aPTT in a dose-dependent manner. Trends were observed for some hematology parameters evaluated (e.g., lymphocyte count, red blood cell count, hematocrit, neutrophil count). However, these trends were considered incidental and of no toxicological importance. Twelve animals were sacrificed early due to issues related to non-specific inflammatory processes. With the exception of vacuolation in various organs (see below), there were no other major histopathological findings.

As N9-GP is a recombinant human protein, animals receiving repeated doses of the product developed antibodies against rFIX that both accelerated clearance of the protein and in some cases, neutralized its pro-coagulant activity. Therefore, long-term, repeat-dose toxicity studies, as well as the standard carcinogenicity bioassay (i.e., two years of daily N9-GP dosing in both rats and mice) were not feasible to conduct.

5.5. PEG Accumulation and Vacuolation Observed in the Nonclinical N9-GP Toxicity Studies

In N9-GP acute nonclinical studies, there was no PEG accumulation or vacuolation noted in any organs or tissues in animal studies receiving one-time doses up to 50-fold greater than the proposed recommended clinical starting dose level of 40 IU/kg. However, in N9-GP repeat-dose nonclinical studies, PEG accumulation and minimal vacuolation were observed in some animals in various tissues and organs and at various time points and dosing regimens. **Table 2** below displays the organs and tissues that had accumulation of PEG and/or vacuole formation in the N9-GP nonclinical toxicity studies.

Table 2. PEG accumulation and vacuolation in organs and tissues for animals in the N9-GP nonclinical toxicity studies.

Study Number	Species	Dose Level(s) (IU/kg)	Dosing Regimen	PEG Accumulation[+]	Vacuolation[++]
1	Wistar Rat	0 200 1000 2000	Single	Not evaluated	None present
2	Cynomolgus Monkey	0 350 1300 3750	Repeat (once weekly for 4 weeks) *5-week recovery period**	Terminal Sacrifice (all dose levels) Choroid plexus connective tissue Choroid plexus epithelial cells Blood in brain blood vessels Skeletal muscle blood vessels	Present in the *liver* of two animals administered 0 and 3750 IU/kg
3	Cynomolgus Monkey	200	Repeat (once weekly for 13 weeks) *5-week recovery period*	None present	None present
4	Wistar Rat	25 200	Repeat (once weekly for 14 days)	Not evaluated	None present
5	Rowett Nude Rat	0 40 1200	Repeat (twice weekly for 6 weeks) *2-week recovery period*	1200 IU/kg Group Choroid plexus connective tissue Choroid plexus epithelial cells Blood in brain blood vessels	▪ Present in the *liver, adrenals, parotid salivary gland, stomach, and lachrymal glands* of animals at all dose levels, including control ▪ Present in the *choroid plexus* of one animal administered 40 IU/kg ▪ Present in the *testes* of one animal administered 1200 IU/kg ▪ Present in the *kidneys* of one animal administered 40 IU/kg

Study Number	Species	Dose Level(s) (IU/kg)	Dosing Regimen	PEG Accumulation[+]	Vacuolation[++]
6	Rowett Nude Rat	0 40 150 600 1200	Repeat (once weekly for 26 weeks) *26-week recovery period for separate high-dose group*	Terminal Sacrifice (all dose levels) Choroid plexus connective tissue Choroid plexus epithelial cells Blood in brain blood vessels Mesenteric lymph nodes Spleen Recovery Sacrifice (1200 IU/kg) Choroid plexus epithelial cells	Terminal Sacrifice - Present in the *mesenteric lymph nodes* and *spleen* of animals at all dose levels, including control - Present in the *adrenals* of two animals administered 0 IU/kg and three animals administered 1200 IU/kg - Present in the *epididymes* of one animal administered 0 IU/kg - Present in the *kidneys* of one animal administered 150 IU/kg - Present in the *lacrimal glands* of one animal administered 0 IU/kg - Present in the *pituitary* of one animal administered 1200 IU/kg - Present in the *mandibular salivary glands* of one animal administered 1200 IU/kg - Present in the *parotid salivary glands* of five animals administered 0 IU/kg and five animals administered 1200 IU/kg - Present in the *tongue* of two animals administered 1200 IU/kg - Present in the *mucosa/submucosa* of one animal administered 1200 IU/kg Recovery Sacrifice - Present in the *liver* of one animal administered 1200 IU/kg - Present in the *mesenteric lymph nodes* of two animals administered 0 and 1200 IU/kg - Present in the *pituitary* of one animal administered 1200 IU/kg

*high-dose animals had only one week of recovery
[+]accumulation of PEG was determined using immunohistochemistry
[++]vacuolation was determined by routine light microscopy

Based on these results, it appears that the dissociation of the PEG moiety from the N9-GP product after *in vivo* administration results in PEG accumulation in macrophages and vacuoles and vesicles within cells. Per the pathology test reports, animals administered clinically higher dose levels of N9-GP had higher incidences of PEG accumulation. Autoradiography studies demonstrated that the amount of radiolabeled N9-GP and PEG decreases over time in the choroid plexus following single IV administration. In addition, rodents appeared more susceptible than monkeys to PEG accumulation and vacuolation. While PEG accumulation was dose- and time-dependent, vacuolation was not. Vacuolation occurred in both control animals and animals administered various dose levels of N9-GP. In some instances where vacuolation was observed, the contents of the vacuoles were evaluated; some vacuoles contained PEG. Vacuolation did not appear to affect the metabolism of N9-GP.

PEG accumulation was detected in the choroid plexus connective tissue of terminally-sacrificed monkeys administered N9-GP at dose levels higher than 200 IU/kg, and the majority of Rowett nude rats administered N9-GP, irrespective of dose level. After four weekly administrations of 350 IU/kg, PEG was detected only in blood within brain blood vessels. Per the expert pathology report, after four weeks of weekly administrations of 1300 and 3750 IU/kg, PEG was detected in the connective tissue and cytoplasm of epithelial cells in the choroid plexus, and in blood within brain blood vessels. These findings did not result in clinically-meaningful effects in the monkeys administered 350 and 1300 IU/kg, as there were no neurological deficits noted based on the neurological/CNS endpoints evaluated. The significance of the finding of PEG in the blood within brain blood vessels remains unknown; however, N9-GP was administered IV; therefore, PEG would be expected in the circulating blood. Following five weeks of recovery in monkeys administered 350 and 1300 IU/kg/week, PEG was not detected in the choroid plexus. However, following one week of recovery, animals administered 3750 IU/kg/week had PEG accumulation in the connective tissue and cytoplasm of epithelial cells in the choroid plexus, and in blood within brain blood vessels. No other brain structures in any of the animals had PEG accumulation. No rFIX was detected in the choroid plexus or any other parts of the brain.

In the 26-week study in Rowett nude rats, PEG was detected in the connective tissue of the choroid plexus, small vesicles in epithelial cells in the choroid plexus, and in blood within brain blood vessels. With electron microscopy (EM), the PEG in the choroid plexus epithelial cells was noted to be confined within intracytoplasmic vesicles. These observations from the EM evaluation were independent of dose level. However, tissue was also examined for the presence of PEG using immunohistochemistry (IHC). According to the IHC evaluation, the number of animals in the 40 IU/kg/week dose level group with detectable PEG in the choroid plexus and blood within brain blood vessels was half that of those in the higher dose level groups (150, 600, and 1200 IU/kg/week). In animals in all dose groups, the pathologist noted that PEG was also detected in the cytoplasm of macrophages, but not in vacuoles, in the mesenteric lymph nodes. No other brain structures (i.e., cerebellum, cerebrum, and midbrain) within the sections examined in any of the animals had PEG accumulation. No rFIX was detected in the choroid plexus or any other parts of the brain.

The clearance of PEG from the choroid plexus, potentially via the CSF, remains unclear.

5.6. 40-kDa PEG Toxicology

Nonclinical toxicity studies were conducted on the 40-kDa PEG moiety used in the manufacturing of N9-GP. Two repeat-dose toxicity studies administering the 40-kDa PEG moiety were conducted in cynomolgus monkeys and Wistar rats.

Monkeys were administered 40-kDa PEG at 45 mg/kg/week (equivalent to ~8437 IU/kg/week of N9-GP) for two or six weeks, or 7 mg/kg/week (equivalent to ~1312 IU/kg/week of N9-GP) for 13 weeks, every other day. The equivalent N9-GP dose levels were derived from a calculation provided in a test report, which stated that 1 mg of FIX protein has 150 units (IU) of activity and for each milligram of rFIX protein 0.8 mg of 40-kDa PEG is attached. Per the test report, these doses represented the maximum possible load of 40-kDa PEG. This study indicated that PEG accumulation and vacuole formation occurred in ependymal cells of the choroid plexus and cortical adrenals for the six-week 45 mg/kg/week dose level group. There were no remarkable systemic toxicities reported in monkeys after acute dosing with the 40-kDa PEG moiety.

Rats were administered 40-kDa PEG at 45 mg/kg/week for two or six weeks, or 117 mg/kg/week (equivalent to ~21937 IU/kg/week of N9-GP) for six weeks, every other day. Per the test report, the 117 mg/kg/week dose level was chosen as a bridge to the maximum total weekly PEG load in previous rat studies conducted with a different, unspecified, PEGylated drug product. This study indicated that PEG accumulated in a dose- and time-dependent manner in the liver, brain, spleen (red pulp), and mesenteric and mandibular lymph nodes of the animals administered 40-kDa PEG for six weeks. Vacuolation occurred in the brain, spleen (red pulp), and to a lesser degree in the mesenteric mandibular lymph nodes and the liver in a dose- and time-dependent manner for the animals administered 40-kDa PEG for six weeks. There were no remarkable toxicities reported in rats after acute dosing with the 40-kDa PEG moiety, although there were changes in food consumption and resulting body weight differences in a dose-dependent manner. Compared to controls, animals receiving either dose of 40-kDa PEG had lower food consumption at six weeks; however, at 45 mg/kg/week there was no effect on food consumption during the first two weeks.

The presence of antibodies to PEG was not evaluated. PEG has long been regarded as non-immunogenic. However, in cases where anti-PEG antibodies have been observed following administration of a PEGylated product, it may be due to the effect of the conjugated immunogenic protein on the PEG moiety [9].

6. Conclusions Regarding the Nonclinical Data

The majority of animals evaluated in the toxicity studies remained healthy until their scheduled sacrifice time point and had no overt signs of toxicity (e.g., irregularities in heart rate, body weight, food consumption, etc.). Any irregularities observed were transient, related to the species of the animal, or related to the development of cross-reacting neutralizing antibodies. These irregularities were not related to the N9-GP. However, the exception to this was monkeys administered 3750 IU/kg/week for four weeks.

Six out of eight monkeys in this dose level group exhibited mild but transient tremors. The cause of these tremors was unknown. Upon microscopic examination, five monkeys in the highest dose level

group (3750 IU/kg/week) had substantial sub-meningeal congestion/hemorrhage in the brain and acute inflammatory cell infiltration in the spinal cord and hemorrhage associated with cellulitis in the skin/subcutis. The hemorrhage observed in these animals was most likely related to the development of cross-reacting neutralizing antibodies, resulting in acquired hemophilia. This conclusion is based on the prolongation of aPTT times in most animals, confirmation of neutralizing antibodies during the recovery period, and the clinical and pathological signs associated with a bleeding tendency (i.e., signs of bruising and/or swelling and hemorrhage). It must also be noted that these animals were administered a dose level almost 100-fold higher than the proposed prophylactic clinical dose level of 40 IU/kg/week. Therefore, the likelihood of development of CNS tremors, cellulitis, and meningeal hemorrhage following chronic administration of N9-GP in humans may be low.

The most notable observations in the histology of the animals in the toxicity studies were the accumulation of PEG in the choroid plexus and vacuolation in various organs. Vacuolation did not appear to be time- or dose-dependent, and was noted in control animals as well as animals dosed with N9-GP. Also, the majority of the observed vacuolation was minimal or slight, per the pathology reports. Furthermore, vacuolation was noted in a sparse number of animals, indicating no pattern. Vacuolation did not appear to cause any adverse structural effects to the cells, affect the metabolism of N9-GP, or result in adverse clinical effects, neurological or otherwise. Therefore, vacuolation may be a less important finding than PEG accumulation.

Accumulation of PEG in the connective tissue and cytoplasm of epithelial cells in the choroid plexus, and in blood within brain blood vessels was one of the most consistent observations in the histology. This observation was observed irrespective of the dose level of N9-GP, though to a lesser extent at the lowest dose administered. Although saturation occurs, PEG metabolism is a continuous process that eliminates PEG in a time- and dose-proportional manner. It is unclear how PEG accumulation in the choroid plexus may affect neurological function. As previously stated, the mechanism of clearance of PEG from the choroid plexus remains unknown. One potential concern is the possibility that PEG could leak into the CSF, exert an osmotic effect, and lead to hydrocephalus due to excessive fluid absorption and increased intra-ventricular pressure [5, 6]. CSF samples were taken from Rowett nude rats administered N9-GP for 26 weeks, but these samples were not analyzed.

There were no neurological deficits observed in the monkeys that were administered 350 or 1300 IU/kg/week for four weeks, even though PEG accumulation was observed in these animals. Although PEG accumulation was observed in the Rowett nude rat studies, no clinical abnormalities were detected. Therefore, it is unclear whether accumulation of PEG in the choroid plexus is clinically important.

Currently, there is no clinical biomarker to assess choroid plexus function. However, the finding of PEG accumulation in the choroid plexus in nonclinical studies raises the issue of the safety of N9-GP in humans.

7. Relevant Literature on Toxicity of PEGylated Proteins

PEGylation is the covalent binding of one or more PEG polymers to a molecule. It is frequently used to improve the half-life and increase stability of proteins [10]. In some cases, PEGylation is used to reduce the immunogenicity of highly immunogenic proteins such as enzymes.

Although PEG is considered an inert polymer, current data indicate that toxicity associated with PEG is present only at very high parenteral doses and is restricted mostly to the kidney [11]. A common and well-documented phenomenon that occurs following administration of PEGylated proteins is vacuolation in macrophages. Vacuolation has also been observed in cells in the choroid plexus.

Many factors may impact the occurrence and degree of cellular vacuolation observed in toxicology studies evaluating PEGylated proteins. These factors include the pharmacological activity of the protein; distribution of protein target; dose level; dosing regimen and duration of administration; distribution of the protein; amount of nonspecific uptake versus specific protein-receptor mediated uptake; potential immunogenicity of the protein; overall molecular weight of the protein; molecular weight of PEG; and types of clearance/removal mechanisms for the protein or PEG [9].

A study by Rudmann *et al*. [4] investigated the effects of unconjugated PEG on vacuolation. Three linear PEG polymers consisting of 10, 20, and 40-kDa molecular weight were administered repeatedly to rats, either daily or every other day, for three months at a dose level of 100 mg/kg. The study demonstrated an inverse relationship between PEG molecular weight and PEG accumulation in the renal tubular epithelial cells, and a direct relationship between PEG molecular weight and PEG accumulation in choroid plexus epithelial cells. These observations were generally limited to 40-kDa PEG. Animals administered this molecular weight PEG also demonstrated vacuolation in macrophages and choroid plexus epithelial cells. The authors concluded that chronic administration of biologics conjugated to PEG ≥40 kDa has a greater potential for vacuolation in macrophages, choroid plexus epithelial cells, and renal tubular epithelial cells [4].

There has been no evidence to-date that vacuolation leads to tissue damage or disruption of functional parameters in any organ. Therefore, vacuolation in cells is generally considered a non-adverse consequence of the natural PEG removal process [12].

8. Overview of the N9-GP Clinical Program

8.1. Summary of the Clinical Studies

8.1.1. Proposed Indications
For use in adults and children with hemophilia B for:
- Control and prevention of bleeding episodes
- Perioperative management
- Routine prophylaxis

8.1.2. Proposed Dosage and Administration

- Control and prevention of bleeding episodes: 40 IU/kg body weight for minor and moderate bleeds, and 80 IU/kg body weight for major bleeds. Additional doses of 40 IU/kg can be given.
- Perioperative management: Pre-operative dose of 40 IU/kg body weight for minor surgery, and 80 IU/kg body weight for major surgery. Consider two repeated doses of 40 IU/kg (in 1-3 day intervals) within the first week after major surgery. Frequency may be extended to once weekly after the first week until bleeding stops and healing is achieved.
- Routine prophylaxis: 40 IU/kg once-weekly

8.1.3. Phase 3 Studies

Study Design

Four clinical trials of N9-GP have been completed. These studies included previously treated children, adolescents, and adults:

Trial ID	Trial Type	Trial Design	Subjects Exposed	Dose
3747	PK, safety, efficacy	Phase 3, single-blind, randomized study for routine prophylaxis and treatment of bleeding episodes	74 adolescent and adult subjects	10 IU/kg: 30 subjects for 52 weeks 40 IU/kg: 29 subjects for 52 weeks On Demand: 15 subjects for 28 weeks
3773	safety, efficacy	Phase 3, open-label, uncontrolled surgery study	13 adolescent and adult subjects	Preoperative: 80 IU/kg Postoperative: 40 IU/kg 24-48 hours after preoperative dose and then as needed
3774	PK, safety, efficacy	Phase 3, open-label, study for routine prophylaxis and treatment of bleeding episodes	25 pediatric subjects	10 IU/kg: none 40 IU/kg: 25 subjects for 52 weeks On Demand: none
3775	safety, efficacy	Extension study, Phase 3, open-label, study for routine prophylaxis and treatment of bleeding episodes	71 adolescent and adult subjects*	10 IU/kg: 21 subjects for 52 weeks 40 IU/kg: 52 subjects for 52 weeks 80IU/kg: 2 subjects for 52 weeks On Demand: 5 subjects for 52 weeks

These subjects were enrolled from Trials 3747 and 3773

Five subjects initially enrolled in 3747, then enrolled in 3775, then enrolled in the surgery trial (3773), and then went back into 3775. The surgery trial (3773) enrolled a total of 7 subjects who participated in at least one of the other trials, and six subjects who did not participate in another trial. Therefore, a total of 105 previously treated patients (PTPs) were exposed to N9-GP in Trials 3747, 3773, 3774, 3775.

The prophylaxis regimens for adolescents and adults were either 10 or 40 IU/kg weekly. The pediatric trial administered 40 IU/kg weekly. Subjects undergoing major surgery received a single bolus injection

of 80 IU/kg N9-GP on the day of surgery. Postoperatively, the subjects were treated with injections of 40 IU/kg.

Study Conduct

Safety information collected included adverse events, laboratory assessments, physical examinations, vital signs, ECGs, and (in Study 3747 only) injection site tolerability.

The Case Report Forms for the initial visit provide examination findings for each organ system, including the neurological system. However, the physical exam findings during the initial visit do not include details of the type of tests performed (e.g., there is no evidence of a comprehensive neurological exam or neurocognitive evaluation). Details of the physical exam findings for subsequent visits are not recorded by organ systems but targeted to identify any new findings judged by the investigator to be an undesirable adverse event. The safety monitoring plan in the N9-GP clinical study protocol did not include a comprehensive examination of the corresponding organ systems (e.g., neurocognitive testing; assessment of renal tubular dysfunction).

Study Results

The primary endpoint of Trials 3747, 3775, and 3774 was to assess the immunogenicity based on the incidence of inhibitory antibodies against FIX, defined as titer ≥ 0.6 Bethesda Unites (BU). For FIX products, acceptability criterion is one subject with inhibitor formation in a sample size of 50 subjects, such that the upper bound of the 95% confidence interval (CI) does not exceed 10.65%. The key secondary endpoints were to a) assess hemostatic efficacy during bleeding episodes using a four point scale (excellent, good, moderate and poor) with excellent and good responses considered as success and b) efficacy during routine prophylaxis as assessed by the number of bleeding episodes measured as annualized bleeding rate (ABR). The primary endpoint of Trial 3773 was to assess hemostatic efficacy following surgery by the investigator or surgeon using a four-point scale rated as excellent, good, moderate or poor response, with excellent and good hemostasis being considered a success.

Trial 3747: No inhibitors were detected during this trial. The mean [standard deviation (SD)] ABR was 4.81 (5.41) and 3.53 (7.41) for the 10 IU/kg and 40 IU/kg dose cohorts, respectively. The median ABR [Interquartile Range (IQR)] was 2.93 [0.99; 6.02] and 1.04 [0; 4], respectively for the 10 IU/kg and 40 IU/kg dose cohorts. The applicant's statistical analysis used 12 bleeding episodes per year as an estimate of the ABR for subjects on demand. An ABR less than 4.8 was considered confirmed if the upper bound of the two-sided 95% CI was below 4.8. Therefore, as shown in the table below, prophylaxis with 40 IU/kg was considered efficacious, whereas efficacy of 10 IU/kg was not demonstrated.

ABR	10 IU/kg Dose	40 IU/kg Dose
Mean (SD)	4.81 (5.41)	3.53 (7.41)
Median (IQR)	2.93 [0.99; 6.02]	1.03 [0; 4]
Poisson Estimate of ABR (95% CI)	4.56 (3.01;6.90)	2.51 (1.42; 4.43)

Trial 3773: A total of 13 surgeries were performed in 13 subjects. The pre-specified criterion for study success was a rating of "good" or "excellent" from at least ten surgeries in 5-10 subjects, including five major surgeries. Upon review of the list of surgeries, nine were judged to be major surgeries and four minor surgeries. The hemostatic effect was rated as "excellent" in 10 and as "good" in 3 of the surgeries. Success in adult surgeries are generally extrapolated to pediatrics.

Trial 3774: No inhibitors were detected during this trial. The mean (SD) ABR for children 0-12 years was 1.42 (1.64) and the Median ABR [IQR] was 1.0 [0.0; 2.1]. For spontaneous bleeds, the mean (SD) ABR was 0.41 (0.95) and the Median ABR [IQR] was 0 [0;0].

Trial 3775: No inhibitors were detected during this trial. The mean (SD) ABR was 2.16 (3.06) and median [IQR] was 1.36 [0; 2.2] for the 10 IU/kg cohort. The mean (SD) ABR was 1.94 (2.78) and median [IQR] was 1.0 [0; 2.0] for the 40 IU/kg cohort. For spontaneous bleeds, the mean (SD) ABR was 1.65 (2.77) and median [IQR] was 1.05 [0; 2.16] for the 10 IU/kg cohort. The mean (SD) ABR was 0.91 (1.93) and median [IQR] was 0.0 [0; 1.0] for the 40 IU/kg cohort.

Safety Listings by System

A total of 647 Adverse Events (AEs) was reported in 98 (85.2%) subjects. The most common adverse events were nasopharyngitis (35 events in 19 [16.5%] subjects), upper respiratory tract infection (20 events in 13 [11.3%] subjects), contusion (27 events in 15 [13.0%] subjects) and cough (24 events in 15 [13.0%] subjects).

There were 29 Nervous System AEs reported in 16 subjects from all three Phase 3 studies. The neurological symptoms included headache, dizziness, sciatic neuralgia, tongue biting, and speech disorder.

Only 3 Renal AEs were reported in 2 subjects from all phase studies. There was a small decrease in mean estimated renal clearance in both adolescent/adults and pediatric subjects.

Urinalysis was performed in the adolescent/adult trial, but was not performed in the pediatric trial. Three subjects tested positive for glucose at one time point; two of these subjects were positive only before dosing. The third subject was positive at the End of Treatment (EOT) visit. There were 27 subjects who tested positive for proteinuria; 12 tested positive prior to dosing with the study drug. There were 15 subjects who had positive test results for protein after dosing with N9-GP. Seven subjects had one positive test (1 at an intermediate visit and 6 at EOT). Six subjects had two positive tests (3 at baseline and EOT, 2 at an intermediate visit and EOT, 1 at baseline and an intermediate visit). One subject had three positive tests. One subject had 4 positive tests (all four visits).

8.1.4. Conclusions on the Clinical Data
No clear safety signal from accumulation of PEGylation was observed in the clinical trials. However, it is unclear whether the monitoring of neurologic function was adequate to detect all clinically important neurologic signs or symptoms. In addition, it is unclear whether the size of the safety database (i.e., number of adult and/or pediatric subjects exposed to the product; duration of follow-up of those subjects) is sufficient to assess the safety of the product.

9. Benefit-Risk Considerations

Benefit Considerations

- Efficacy of the product with a median [IQR] ABR of 1.03 [0;4] in adolescents and adults and a median [IQR] ABR of 1.0 [0.0; 2.1] in children.

- Decreased dosing frequency for prophylactic treatment (i.e., 40 IU/kg once weekly)

Risk Considerations

- Per the pre-clinical studies, the choroid plexus developed PEG accumulation and vacuolation. The neurological monitoring during the clinical trials may have been insufficient to detect early signs of dysfunction in these systems.

- In the absence of pre-clinical data to confirm the reversibility of the pathological findings and the duration of monitoring necessary to assess the long-term risks of PEG accumulation, and vacuolation, in the target tissues, the adequacy of the safety monitoring period in the clinical studies is unclear.

- If PEG accumulation, and vacuolation, were to occur in humans, then the developing neurological system may be at the greatest risk for associated adverse events. Therefore, the most vulnerable population may be pediatric patients.

- The long-term effects of PEG accumulation, and vacuolation, are unknown in the elderly population, who may be particularly susceptible to renal dysfunction and cognitive impairment. The median age in Study 3747 for subjects receiving 40 IU/kg (routine prophylaxis in adults and adolescents) was 26 years. Only two subjects at least age 60 years (i.e., one subject age 60 and one subject age 65 years) were included in the study; therefore, there is a paucity of data on N9-GP use in older adults.

- The duration of administration of the prophylactic regimen was 52 weeks in Study 3747. Therefore, there are few data on the safety of N9-GP when administered for longer periods, as would be expected if the product is used for chronic prophylaxis.

Available Therapies for Hemophilia B

- Of the recombinant FDA-approved Factor IX products, two products are administered weekly for use in adults and children. These products are also approved for treatment of peri-operative and acute bleeding.

10. DRAFT Questions for the Committee

[The following are draft questions which may be revised prior to the Advisory Committee meeting.]

Following repeat intravenous administration of N9-GP to nude rats and immune-competent nonhuman primates (NHPs), accumulation of PEG was observed in the choroid plexus. Vacuolation was observed in the choroid plexus of nude rats. In addition, some NHPs administered a high dose of N9-GP exhibited transient tremors. The relevance of these findings to the function of the choroid plexus and the risk of neurological adverse effects is unknown.

The proposed indication for the PEGylated recombinant coagulation factor IX (BLA 125611) is for use in adults and children with hemophilia B for:

- Control and prevention of bleeding episodes: 40 IU/kg body weight for minor and moderate bleeds, and 80 IU/kg body weight for major bleeds. Additional doses of 40 IU/kg can be given.

- Perioperative management: Pre-operative dose of 40 IU/kg body weight for minor surgery, and 80 IU/kg body weight for major surgery. Consider two repeated doses of 40 IU/kg (in 1-3 day intervals) within the first week after major surgery. Frequency may be extended to once weekly after the first week until bleeding stops and healing is achieved.

- Routine prophylaxis: 40 IU/kg once-weekly.

1. Please discuss the clinical significance, if any, of the preclinical findings.

2. Please discuss the nature and level of your concerns, if any, regarding the safety of the product in different age populations: e.g., from birth to < 6 years, 6 years to < 12 years, 12 years to 17 years, adults, and older adults.

3. Considering the findings from the toxicology and clinical studies, please discuss whether the data provide sufficient evidence of the safety of the product for: (1) Intermittent use (i.e., for perioperative management and control of bleeding episodes); (2) Chronic, and possibly life-long, use (i.e., routine prophylaxis).

4. Please discuss any clinical or laboratory assessments (including, but not limited to, assessments of neurologic function), either short-term or long-term, that you would recommend to help ensure the safety of patients (or study subjects) who receive the product.

5. Please discuss your recommendations, if any, for additional preclinical or clinical studies, either premarketing or post-marketing, to support the safety of the product. For example, please discuss the clinical examinations and laboratory studies, the duration of follow-up, the inclusion or exclusion of specific age groups, and safety endpoints that you would recommend for any subsequent clinical trials or post-marketing studies.

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