

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research (CBER)
Office of Tissues and Advanced Therapies (OTAT)
Division of Clinical Evaluation and Pharmacology/Toxicology (DCEPT)
Pharmacology/Toxicology Branch 2 (PTB2)

SECONDARY REVIEW MEMO

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TO: The Record

FROM: Becky Robinson-Zeigler, PhD, Chief, PTB2

THROUGH: Tejashri Purohit-Sheth, MD, Director, DCEPT

PRODUCT: REBINYN™; coagulation factor IX (recombinant), glycoPEGylated; N9-GP

APPLICANT: Novo Nordisk A/S

PROPOSED INDICATION: Indicated for use in adults and children with hemophilia B for: on-demand treatment and control of bleeding episodes; perioperative management of bleeding; and routine prophylaxis to reduce the frequency of bleeding episodes.

EXECUTIVE SUMMARY:

The nonclinical program for N9-GP consisted of multiple proof-of-concept (POC) activity and safety studies. Safety pharmacology studies were conducted in murine and canine models of hemophilia, and in healthy cynomolgus monkeys. Pharmacokinetic (PK) studies included general PK and absorption, distribution, metabolism, and excretion (ADME) studies. Autoradiography studies were conducted to determine the distribution of N9-GP and the 40-kDa poly(ethylene glycol) (PEG) moiety. Local tolerance studies were also conducted in healthy rabbits.

For their definitive nonclinical safety studies, the applicant conducted single and repeat-dose toxicity studies in healthy, immune-competent Wistar rats and cynomolgus monkeys, and in immune-deficient, Rowett nude rats (**Table 1**). These definitive nonclinical safety studies evaluated the proposed prophylactic clinical dose level of 40 IU/kg and dose levels ranging from five-fold to almost 100-fold higher than the clinical dose. Finally, the applicant conducted a second set of studies evaluating the toxicity of 40-kDa PEG alone in healthy cynomolgus monkeys and Wistar rats. All studies administered N9-GP using the intravenous (IV) route of administration, which is also the proposed clinical route of administration.

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) <i>5-week recovery period*</i>	4 weeks post-first administration (Terminal sacrifice) 5 weeks post-last administration (Recovery sacrifice)
3	Cynomolgus Monkey	200	Repeat (once weekly for 13 weeks) <i>5-week recovery period</i>	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) <i>2-week recovery period</i>	7-8 weeks post-first administration
6	Rowett Nude Rat	0 40 150 600 1200	Repeat (once weekly for 26 weeks) <i>26-week recovery period for a separate high-dose group only</i>	26 weeks post-first administration (Terminal sacrifice) 26 weeks post-last administration (Recovery sacrifice)

**high-dose animals had only one week of recovery*

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 activated partial thromboplastin time (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 (**Table 2**). 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 accumulation of PEG.

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. 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.^{1,2} However, there were no neurological deficits observed in 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. CSF samples were taken from Rowett nude rats administered REBINYN for 26 weeks, but these samples were not analyzed. Therefore, it is unclear whether accumulation of PEG in the choroid plexus is clinically important for patients using REBINYN for prophylaxis.

This safety issue was presented to the Blood Products Advisory Committee (BPAC) on April 4, 2017. The committee concluded that while accumulation of PEG in the choroid plexus is a concern, additional nonclinical studies premarket would not be necessary. Post-market monitoring of potential neurological adverse events would suffice to permit approval of the prophylaxis indication. The committee had no concerns related to on-demand or perioperative use of REBINYN.

Based on the opinions from members of the advisory committee and in light of the aforementioned nonclinical findings, the label for REBINYN was revised to incorporate recommendations for monitoring of neurologic dysfunction and provide a brief summary in *Section 13.2 Animal Toxicology and/or Pharmacology* of the major findings from the toxicology studies (i.e., accumulation of PEG in the choroid plexus). *Section 8.1 Pregnancy* and *Section 8.2 Lactation* of the label were revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14), and align with the provisions in the other recently licensed recombinant human coagulation factors for hemophilia B.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

The nonclinical data support the licensure application for the indications of on-demand treatment and control of bleeding episodes, and perioperative management of bleeding.

¹ Gardner, W.J., D.K. Spitler, and C. Whitten, *Increased intracranial pressure caused by increased protein content in the cerebrospinal fluid; an explanation of papilledema in certain cases of small intracranial and intraspinal tumors, and in the Guillain-Barre syndrome*. N Engl J Med, 1954. **250**(22): p. 932-6.

² Krishnamurthy, S., et al., *Intraventricular infusion of hyperosmolar dextran induces hydrocephalus: a novel animal model of hydrocephalus*. Cerebrospinal Fluid Res, 2009. **6**: p. 16.

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) <i>5-week recovery period*</i>	<u>Terminal Sacrifice (all dose levels)</u> Choroid plexus connective tissue Choroid plexus epithelial cells Blood in brain blood vessels Skeletal muscle blood vessels	Present in the <i>liver</i> of two animals administered 0 and 3750 IU/kg
3	Cynomolgus Monkey	200	Repeat (once weekly for 13 weeks) <i>5-week recovery period</i>	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) <i>2-week recovery period</i>	<u>1200 IU/kg Group</u> Choroid plexus connective tissue Choroid plexus epithelial cells Blood in brain blood vessels	<ul style="list-style-type: none"> Present in the <i>liver, adrenals, parotid salivary gland, stomach, and lachrymal glands</i> of animals at all dose levels, including control Present in the <i>choroid plexus</i> of one animal administered 40 IU/kg Present in the <i>testes</i> of one animal administered 1200 IU/kg Present in the <i>kidneys</i> 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	<u>Terminal Sacrifice</u> Choroid plexus connective tissue Choroid plexus epithelial cells Blood in brain blood vessels Mesenteric lymph nodes Spleen <u>Recovery Sacrifice (1200 IU/kg)</u> Choroid plexus epithelial cells	<u>Terminal Sacrifice</u> <ul style="list-style-type: none"> Present in the <i>mesenteric lymph nodes</i> and <i>spleen</i> of animals at all dose levels, including control Present in the <i>adrenals</i> of two animals administered 0 IU/kg and three animals administered 1200 IU/kg Present in the <i>epididymes</i> of one animal administered 0 IU/kg Present in the <i>kidneys</i> of one animal administered 150 IU/kg Present in the <i>lacrimal glands</i> of one animal administered 0 IU/kg Present in the <i>pituitary</i> of one animal administered 1200 IU/kg Present in the <i>mandibular salivary glands</i> of one animal administered 1200 IU/kg Present in the <i>parotid salivary glands</i> of five animals administered 0 IU/kg and five animals administered 1200 IU/kg Present in the <i>tongue</i> of two animals administered 1200 IU/kg Present in the <i>mucosa/submucosa</i> of one animal administered 1200 IU/kg <u>Recovery Sacrifice</u> <ul style="list-style-type: none"> Present in the <i>liver</i> of one animal administered 1200 IU/kg Present in the <i>mesenteric lymph nodes</i> of two animals administered 0 and 1200 IU/kg Present in the <i>pituitary</i> 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