

**ORIGINAL ARTICLE**

# Fixed doses of N8-GP prophylaxis maintain moderate-to-mild factor VIII levels in the majority of patients with severe hemophilia A

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**Abstract**

**Background:** N8-GP is an extended half-life recombinant factor VIII (FVIII) developed for prophylaxis and treatment of bleeds in patients with hemophilia A.

**Objective:** To assess pharmacokinetic (PK) characteristics of N8-GP in previously treated patients with severe hemophilia A, model the time spent at hemophilia thresholds of  $\geq 1$  and  $\leq 5$  IU/dL (moderate) or  $>5$  IU/dL (mild) FVIII levels during N8-GP prophylaxis, and investigate the relationship between N8-GP half-life and von Willebrand factor (vWF).

**Methods:** PK assessments were obtained from patients with severe hemophilia A (FVIII  $< 1$  IU/dL) participating in 4 clinical trials: pathfinder 1 (20-60 years); pathfinder 2 (12-17 and  $\geq 18$  years); pathfinder 5 (0-11 years), and pathfinder 7 (25-71 years). All PK profiles were assessed after washout and considered single-dose PK profiles. Pre- and postdose FVIII activity at steady state was measured at all visits.

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**Results:** From 69 patients, 108 PK profiles of N8-GP 50 IU/kg were assessed. Adults/adolescents received 50 IU/kg every 4 days, achieving mean trough levels of 3.0 IU/dL (95% confidence interval, 2.6-3.5, adults) and 2.7 IU/dL (1.8-4.0, adolescents). Children received 60 IU/kg twice weekly, leading to mean trough levels of 1.2 IU/dL (0.8-1.6, 0- to 5-year-olds) and 2.0 IU/dL (1.5-2.7, 6- to 11-year-olds). PK modeling predicted children dosed every 3 days and adults/adolescents dosed every 3 to 4 days would maintain FVIII levels >5 and >1 IU/dL for >80% and 100% of the time, respectively. N8-GP half-life correlated linearly with von Willebrand factor levels in adults/adolescents, less in children.

**Conclusions:** Prophylaxis with fixed intervals (Q4D/twice weekly) and fixed weight-based dosing (50/60 IU/kg) ensured >1 IU/dL FVIII trough levels in both adults and children.

#### KEYWORDS

factor VIII, hemophilia A, pharmacokinetics, recombinant, von Willebrand factor

#### Essentials

- N8-GP is an extended half-life recombinant factor VIII product for the treatment and prophylaxis of bleeds in hemophilia A.
- Measured mean trough levels were 1.2 and 3.0 IU/dL in children and adults/adolescents, respectively.
- Q3/4D dosing achieved >5/>1 IU/dL factor VIII activity for 94.9/100% (adults/adolescents) and 72.3/97.9% (children) of the time.
- N8-GP half-life correlated linearly with von Willebrand factor levels but was less pronounced in children vs. adults/adolescents.

## 1 | INTRODUCTION

In patients with hemophilia A, the standard of care is prophylaxis with factor VIII (FVIII) concentrates.<sup>1</sup> Compared with on-demand administration, prophylactic administration of FVIII products reduces the frequency of bleeds and thus prevents or slows the progression of joint damage.<sup>1-3</sup> Increased time per week spent with low FVIII levels (<1 IU/dL) during prophylaxis is known to be a determinant of breakthrough bleeding risk, especially in children.<sup>4,5</sup>

Prophylaxis with a standard half-life (SHL) FVIII product requires intravenous (i.v.) injection between 2 and 3 times a week.<sup>1</sup> However, even this frequent dosing schedule cannot always maintain FVIII trough levels  $\geq 1$  IU/dL in all patients, which increases the risk of spontaneous bleeding.<sup>4</sup> Furthermore, such frequent dosing regimens can be burdensome for patients and caregivers and difficult to integrate into daily life,<sup>6,7</sup> presenting a barrier to adherence and potentially compromising long-term treatment outcomes.

Turoctocog alfa pegol (N8-GP, Novo Nordisk, Bagsværd, Denmark), a glycoPEGylated extended half-life (EHL) recombinant FVIII (rFVIII) product, requires less frequent dosing than SHL FVIII products.<sup>8-10</sup> In N8-GP, site-directed glycoPEGylation of rFVIII has been used to extend the half-life of FVIII without impairing its hemostatic activity.<sup>11</sup> Its efficacy and safety have been documented in phase 3 clinical trials in all age groups.<sup>8,9</sup> In the pathfinder 1 trial, N8-GP was found to have a half-life 1.6 times longer than that of plasma-derived or SHL rFVIII products; it also demonstrated

dose-linear pharmacokinetics (PK) in the dose range investigated (25, 50, and 75 IU/kg).<sup>10</sup> In addition to the efficacy and safety, the PK of N8-GP has been further investigated in adults/adolescents and children with severe hemophilia A in pathfinder 2 and 5, respectively.<sup>8,9</sup> pathfinder 7 (Table 1) was conducted to investigate PK and safety in adults/adolescents using N8-GP produced by an optimized manufacturing process at a new manufacturing unit.

One factor influencing the half-life of SHL FVIII products is the tight, noncovalent association of circulating FVIII with the patient's endogenous von Willebrand factor (vWF), which maintains the stability of FVIII and delays its degradation and clearance.<sup>12</sup> As such, FVIII's half-life has been shown to correlate positively with patients' endogenous vWF levels.<sup>13</sup> Current evidence suggests that N8-GP has similar affinity for vWF as nonPEGylated FVIII *in vivo*.<sup>14</sup> In mice lacking endogenous vWF, the half-life of N8-GP was approximately half that in mice with endogenous vWF, but ~20 times that of nonPEGylated FVIII in vWF-deficient animals.<sup>14</sup> Other PEGylated EHL products also show prolonged FVIII half-life *in vivo*.<sup>15,16</sup>

In this paper, we describe an analysis of the single-dose PK characteristics of N8-GP in previously treated patients of all ages with severe hemophilia A across all studies. In addition, steady-state FVIII activity profiles were modeled for different age groups and prophylaxis regimens used in the pivotal studies based on single-dose PK data collected during 4 clinical trials and compared with observed trough levels. This allowed prediction of the time spent

**TABLE 1** Overview of evaluable PK profiles from patients dosed with 50 IU/kg N8-GP

Age range (y)	pathfinder 1 (NCT01205724)		pathfinder 2 (NCT01480180)		pathfinder 5 (NCT01731600)		pathfinder 7 (NCT02920398)		Total		Median (range) baseline age
	Patients	Profiles	Patients	Profiles	Patients	Profiles	Patients	Profiles	Patients	Profiles	
0-5	—	—	—	—	13	13	—	—	13	13	4.0 (1-5)
6-11	—	—	—	—	11	11	—	—	11	11	10.0 (6-11)
12-17	—	—	3	5	—	—	—	—	3	5	13.0 (13-14)
≥18	8	8	20	35	—	—	18 <sup>a</sup>	36	42	79	32.5 (20-71)

Notes: Values are given for the numbers of patients with evaluable profiles and the numbers of evaluable profiles. Only patients/profiles that contributed to primary PK analysis were included. Patients may contribute PK profiles to >1 trial. PK profiles were excluded from analysis due to: predose FVIII activity >5 IU/dL (n = 3), presence of inhibitors before dosing (n = 2), incomplete profiles (n = 2), incorrect dose administration (n = 1), unexpectedly low FVIII exposure compared with other visits (n = 1), inconclusive evidence of a single-dose i.v. profile (n = 1), dosing out of compliance (n = 1), or discrepancy between the 1-stage and chromogenic assays (n = 2).

Abbreviations: FVIII, factor VIII; i.v., intravenous; PK, pharmacokinetic.

<sup>a</sup>This includes 4 patients who were also in the pathfinder 2 trial.

with FVIII activity levels above the mild (FVIII > 5 IU/dL) and moderate (FVIII ≥ 1 and ≤5 IU/dL) hemophilia thresholds during prophylaxis with both N8-GP and with previous FVIII products. The correlation between the half-life of FVIII activity and endogenous vWF plasma levels following dosing with N8-GP was also explored.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Previously treated males of all ages with severe congenital hemophilia A (FVIII < 1 IU/dL), no history of FVIII inhibitors, and body weight (BW) ≥ 10 kg were eligible for inclusion in 4 clinical trials exploring the PK of N8-GP (pathfinder 1<sup>8-10</sup> and 7). Prior to participation, patients were required to have ≥150 exposure days (patients aged ≥6 years) or ≥50 exposure days (patients aged ≤5 years) to any FVIII product.

### 2.2 | Trial design

Four multinational clinical trials have investigated the PK of N8-GP as part of the pathfinder clinical trial program. In pathfinder 1, a first-in-human, dose-escalation trial (phase 1), adults (aged ≥18 years) received a single i.v. bolus dose of N8-GP.<sup>10</sup> The phase 3 pathfinder 2 trial evaluated N8-GP prophylaxis in adults or adolescents aged ≥12 years, administered as a single i.v. bolus at 50 IU/kg every fourth day (Q4D) or every 3 to 4 days (twice weekly).<sup>8</sup> In the phase 3 pathfinder 5 trial, children aged <12 years (younger cohort, 0-5 years; older cohort, 6-11 years) received N8-GP prophylaxis at ~60 IU/kg twice weekly. An increase in dose frequency from twice weekly to every third day was permitted at the investigators' discretion (based on bleeding pattern).<sup>9</sup> Finally, pathfinder 7<sup>17</sup> investigated the PK of N8-GP manufactured by the same process as that utilized in the phase 3 trials and also by an optimized process at a new production site. Eligible patients for pathfinder 7 were those who had previously

participated in pathfinder 2; in pathfinder 7, patients received N8-GP as a single i.v. bolus dose at 50 IU/kg from both production processes.

All trials were approved by independent ethics committees and local institutional review boards and were conducted in accordance with the Declaration of Helsinki<sup>18</sup> and Good Clinical Practice guidelines.<sup>19</sup> Written informed consent was obtained from all patients prior to any trial-related activity. The pathfinder 1, 2, 5, and 7 trials are registered at [www.clinicaltrials.gov](http://www.clinicaltrials.gov) as NCT01205724, NCT01480180, NCT01731600, and NCT02920398, respectively.

### 2.3 | Outcomes evaluated

The clinical outcomes of pathfinder 1 and the main phases of pathfinder 2 and 5 have already been described.<sup>8-10</sup> The current analysis presents the following: combined single-dose PK characteristics of 50 IU/kg N8-GP from the pathfinder 1, 2, 5, and 7 trials; a PK model used to predict the amount of time that FVIII activity levels would be >1 and >5 IU/dL during prophylactic treatment with N8-GP; observed FVIII trough levels at steady state compared with the predicted values; and evaluation of the correlation between the half-lives of N8-GP and patients' previous FVIII products with their endogenous vWF levels.

### 2.4 | PK evaluation

All PK sessions were performed after a washout period and were therefore considered single-dose PK sessions. In pathfinder 1, 2, and 7, single doses of N8-GP 50 IU/kg were administered with a minimum washout of 4 or 7 days before the PK session with previous FVIII product or N8-GP doses, respectively. In pathfinder 5, the washout period was 3.5 days for N8-GP and 3 days for the patients' previous FVIII product.

In pathfinder 1, single-dose PK assessments of N8-GP 50 IU/kg were undertaken in 8 patients using samples collected at baseline immediately prior to N8-GP injection and then at 30 minutes and 1,

4, 8, 12, 24, 30, 48, 72, 96, 120, 144, and 168 hours after dosing.<sup>10</sup> In pathfinder 2, a subgroup of 24 adults/adolescents underwent single-dose PK assessments using samples collected before dosing and 30 minutes and 1, 4, 12, 24, 48, 72, and 96 hours after dosing following their first dose; PK assessments were repeated after a washout period at ~28 weeks (visit 7).<sup>8</sup> In pathfinder 5, PK samples were collected from 27 children before dosing and 1, 6, 24, 30, 72, and 96 hours after the first dose.<sup>9</sup> In addition, patients' PK of previous FVIII product (using fewer time points than in adults) was evaluated before starting N8-GP. In pathfinder 7, PK assessments of N8-GP 50 IU/kg were collected from 21 patients before dosing and 30 minutes and 1, 4, 6, 8, 24, 28, 48, 72, and 96 hours after dosing.

## 2.5 | Trough levels of FVIII

Trough activity of FVIII was defined as the FVIII activity measured immediately before the next dose is given (predose) and was expected to be the lowest activity observed. In pathfinder 2, predose FVIII activity was measured at all visits during prophylaxis for adults/adolescents who received N8-GP 50 IU/kg every 4 days, and for children who received 60 (50-75) N8-GP IU/kg twice weekly in pathfinder 5. Only FVIII activity data measured in samples drawn between 2 and 6 days after the last N8-GP dose and  $\geq 8$  days after the last bleeding episode were included in the analysis for FVIII trough activity.<sup>8</sup> Similar to pathfinder 2, predose FVIII activity was measured at all visits in pathfinder 5. Samples for evaluation of predose FVIII activity (troughs) in children were collected for both N8-GP and for the previous FVIII product.<sup>9</sup>

## 2.6 | Evaluation of vWF

Baseline plasma vWF antigen levels were determined from samples taken at the screening visit (ie, very close to the time of PK assessment) for patients in pathfinder 2, 5, and 7.

## 2.7 | Laboratory measurements

All laboratory parameters were analyzed centrally. As previously described,<sup>8-10</sup> PK assessments were based on FVIII activity in plasma as measured using a chromogenic assay (Chromogenix Coamatic Factor VIII; DiaPharma Group, Inc., West Chester, OH, USA) and a Coasys Plus C coagulation analyzer (Roche Diagnostics, Indianapolis, IN, USA). The assay calibrator was a noncommercially available, N8-GP product-specific standard, the FVIII activity of which had been assessed relative to the World Health Organization International Standard FVIII concentrate with the chromogenic assay. In addition, a normal human plasma standard was used to measure FVIII activity in patients administered with previous FVIII products. vWF antigen was measured at baseline by an enzyme-linked immunosorbent assay (with 2 specific antibodies against vWF; Technozym vWF:Ag ELISA, Technoclone GmbH, Vienna, Austria).

## 2.8 | PK modeling

Single-dose PK parameters (ie, clearance [CL] and volume of distribution at steady state [ $V_{ss}$ ]) were used to model steady-state PK profiles by means of a 1-compartment distribution model with first-order elimination, allowing for between-patient variation and assuming linear kinetics. The single-dose PK profiles predicted mean CL and  $V_{ss}$  for each age group. FVIII levels declined mono-exponentially after dosing and are well described by a 1-compartment model.<sup>20</sup> The PK parameter estimates were then used to predict steady-state PK and FVIII activity profiles. In children, where the elimination rate constant could not be derived due to limited numbers of FVIII activity measurements above the lower limit of quantification (LLOQ), a population-based approach was used. Two steady-state values for Q3/4D dosing reflect that the peak and trough are not similar when the dosing intervals are not similar (ie, 3 and 4 days between dosing).

## 2.9 | Observed FVIII activity levels at steady state

Pre- and postdose FVIII activity at steady state was measured at all visits in a total of 175 adults/adolescents in pathfinder 2 and 68 children in pathfinder 5 receiving N8-GP prophylaxis. The observed FVIII trough levels at steady state were compared with the predicted values.

## 2.10 | Statistical analysis methods

PK parameters were derived from observed PK profiles by means of noncompartmental methods and presented using summary statistics.

The PK parameters assessed were plasma FVIII activity at 30 minutes (adults/adolescents only) and at 60 minutes (adults/adolescents and children), incremental recovery (IR) at 30 minutes (adults/adolescents only) and at 60 minutes (adults/adolescents and children), CL, terminal half-life ( $t_{1/2}$ ), and area under the plasma concentration-time curve from time 0 extrapolated to infinity ( $AUC_{0-\infty}$ ). Plasma FVIII activities below the LLOQ of 0.9 IU/dL were set to  $0.5 * 0.9 = 0.45$  IU/dL.

Mean observed FVIII trough levels (with 95% confidence intervals [CIs]) were calculated using a mixed model of plasma activities, with age group as fixed effect and patient as a random effect. The relationship between FVIII activity half-life values and vWF antigen from individual patients was evaluated using linear regression analyses with 95% CI.

## 3 | RESULTS

### 3.1 | Patients

In total, 108 single-dose PK profiles of N8-GP 50 IU/kg were assessed from 69 unique patients, including 24 aged <12 years (Table 1). Several patients participated in >1 PK trial and some had 3 to 4 PK profiles. The numbers of patients included in the analysis

according to age group were 13, 11, 3, and 42 for those aged 0 to 5, 6 to 11, 12 to 17, and  $\geq 18$  years, respectively (Table 1).

### 3.2 | Single-dose PK

In adults, the mean peak FVIII activity measured after a single dose of 50 IU/kg was 134.4 IU/dL at 30 minutes, falling to 3.5 IU/dL after 96 hours. The mean peak FVIII activity in children was 101.2 IU/dL (0–5 years) and 119.6 IU/dL (6–11 years) at 60 minutes (Table 2) (Figure 1A, B). FVIII activity was higher in adolescents and adults than in children. Both younger (0–5 years) and older (6–11 years) children tended to have a shorter  $t_{1/2}$  and lower peak FVIII activity levels than adults/adolescents (Figure 1B); IR also appeared to be lower, and dose-adjusted CL higher, in children than in adults/adolescents, with a trend toward increasing  $t_{1/2}$  and IR, and CL decreasing with age (Table 2).

The results of the single-dose PK evaluation are summarized by age in Table 2 and shown in Figure 1A and B. Figure 1A presents PK profiles from the different trials and demonstrates that the PK profiles of N8-GP are similar across time and trials in adults/adolescents. Figure 1B demonstrates that the PK profiles of N8-GP are similar in younger and older children (Figure 1B). PK profiles in adults/

adolescents were similar after the first dose and after 28 weeks' treatment (Figure 1A), showing consistent PK over time.

Of the 45 unique patients aged  $>12$  years with evaluable PK profiles after dosing with 50 IU/kg in pathfinder 1, 2, and 7 (only patients/profiles that contributed to the primary PK analysis were included), 1 had a body mass index (BMI) of 16.9 kg/m<sup>2</sup>, 27 were within the normal BMI range (18.5–24.9 kg/m<sup>2</sup>), 12 had a BMI of 25–29.9 kg/m<sup>2</sup>, and 6 had a BMI  $\geq 30$  kg/m<sup>2</sup> (obesity range) at baseline. One of these patients appeared in 2 BMI groups due to an observed change in BMI from participation in pathfinder 2 to pathfinder 7 (Table S1).

### 3.3 | Observed/measured mean FVIII trough activity levels

Mean FVIII trough activity levels during prophylaxis in adults/adolescents were 2.7 IU/dL (95% CI, 1.8–4.0) and 3.0 IU/dL (95% CI, 2.6–3.5) in adolescents (12–17 years) and adults ( $\geq 18$  years), respectively, when dosed with N8-GP 50 IU/kg Q4D. Mean FVIII trough activity levels were 1.2 IU/dL (95% CI, 0.8–1.6) and 2.0 IU/dL (95% CI, 1.5–2.7) in children (ages 0–5 and 6–11 years, respectively) when dosed with N8-GP  $\sim 60$  IU/kg twice weekly.

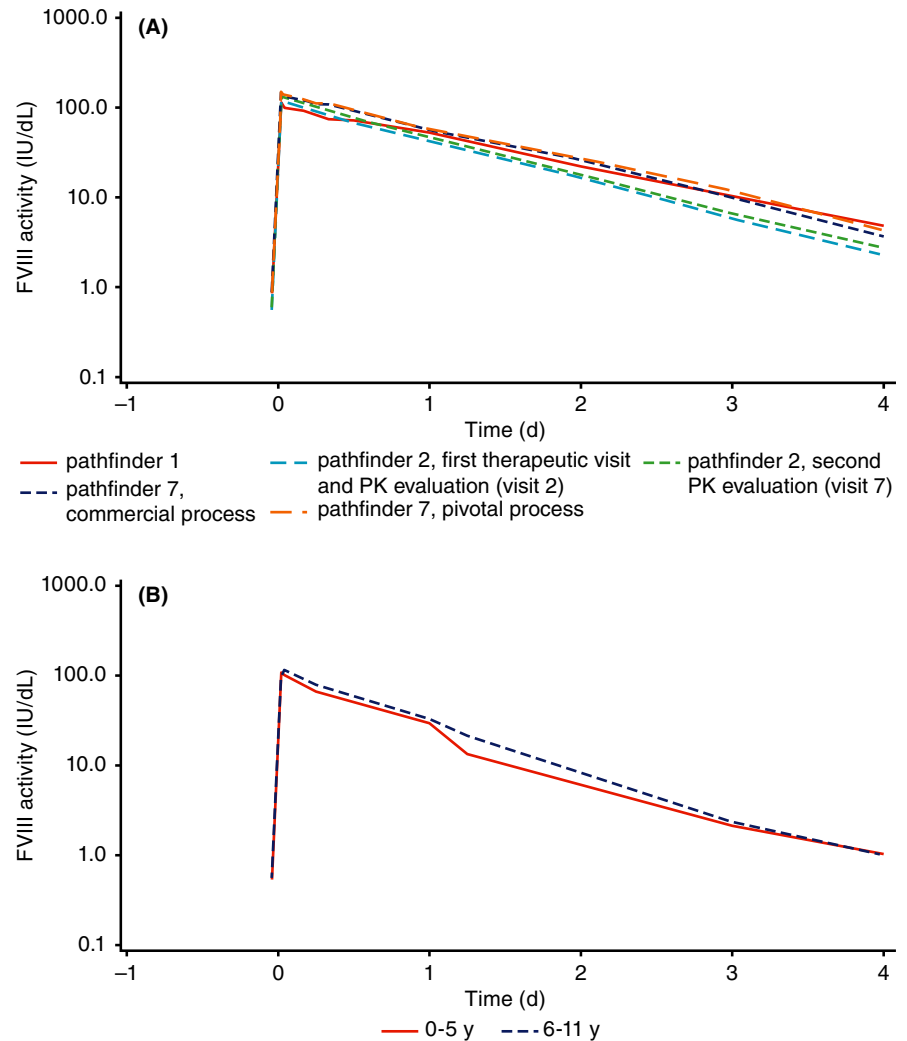
PK parameter	0-5 y (n = 13)	6-11 y (n = 11)	12-17 y (n = 3)	$\geq 18$ y (n = 42)
FVIII activity at 30 min (IU/dL) <sup>a</sup>				
Number of profiles	–	–	5	79
Geometric mean (CV %)	–	–	133.2 (8.7)	134.4 (23.3)
FVIII activity at 60 min (IU/dL)				
Number of profiles	13	11	5	79
Geometric mean (CV %)	101.2 (28.3)	119.6 (25.0)	123.9 (6.4)	124.3 (23.8)
Incremental recovery at 30 min ([IU/dL]/[IU/kg]) <sup>a</sup>				
Number of profiles	–	–	5	79
Geometric mean (CV %)	–	–	2.79 (12.19)	2.63 (22.09)
Incremental recovery at 60 min ([IU/dL]/[IU/kg])				
Number of profiles	13	11	5	79
Geometric mean (CV %)	1.80 (29.14)	1.99 (24.91)	2.59 (8.58)	2.43 (23.43)
Clearance (mL/h/kg)				
Number of profiles	13	11	5	79
Geometric mean (CV %)	2.6 (44.7)	2.4 (39.6)	1.5 (42.8)	1.4 (32.1)
Terminal half-life (h)				
Number of profiles	13	11	5	79
Geometric mean (CV %)	13.6 (20.4)	14.2 (26.1)	15.8 (43.2)	19.9 (34.2)
AUC <sub>0–inf</sub> (IU/h/dL)				
Number of profiles	13	11	5	79
Geometric mean (CV %)	2147 (47)	2503 (42)	3100 (44)	3686 (35)

**TABLE 2** Single-dose PK parameters of N8-GP (50 IU/kg)

Abbreviations: AUC<sub>0–inf</sub>, area under the curve from time 0 extrapolated to infinity; CV, coefficient of variation; FVIII, factor VIII; n, number of patients; PK, pharmacokinetic.

<sup>a</sup>The first sampling in children aged  $<12$  y was after 1 h, in accordance with the European Medicines Agency guideline for FVIII products.<sup>21</sup>

**FIGURE 1** PK profiles of FVIII activity (IU/dL) after a single dose of 50 IU/kg N8-GP (A) in patients aged  $\geq 12$  y in pathfinder 1, 2, and 7, by trial; and (B) in patients aged  $< 12$  y in pathfinder 5. Data are  $\pm$ SEM; FAS, full analysis set; FVIII, factor VIII; PK, pharmacokinetic; SEM, standard error of the mean



### 3.4 | PK predictions

The predicted PK parameters at steady state, times to FVIII levels of 1 or 5 IU/dL, and percentage of time with FVIII levels  $> 1$  or  $> 5$  IU/dL are shown in Table 3. Predicted trough levels after 96 hours were close to levels observed/measured during prophylaxis in adults/adolescents and children: In adolescents/adults dosed with N8-GP 50 IU/kg Q4D, it was 3.5 IU/dL; in children aged 0-11 years treated with N8-GP  $\sim 60$  IU/kg every 3 or 4 days (Q3/4D), it was 2.8 (after 3 days)/0.8 (after 4 days) IU/dL (Table 3).

### 3.5 | Modeling protection across the week

Patients aged  $\geq 12$  years dosed Q3/4D or Q4D with 50 IU/kg N8-GP were predicted to have FVIII activity  $> 5$  IU/dL for 94.9% and 90.0% of the time, respectively, with FVIII activity  $> 1$  IU/dL for 100% of the time with either dosing regimen (Table 3).

For patients aged  $< 12$  years dosed with  $\sim 60$  IU/kg, FVIII activity of  $> 5$  IU/dL was also predicted for most of the interval with Q3/4D and Q3D dosing (72.3% and 84.6%, respectively) (Table 3). Furthermore, FVIII activity  $> 1$  IU/dL was expected 97.9% of the time

with the Q3/4D pediatric regimen and for the entire dosing interval for the Q3D (Table 3).

Comparing FVIII levels on a Q3D regimen with either N8-GP or with the patient's previous SHL rFVIII or plasma-derived FVIII (pd-FVIII) product, the overall trend with N8-GP was to have longer periods of time (between 1 and 2 days longer) with FVIII activity levels  $> 1$  and  $> 5$  IU/dL in all age groups (Figure 2A,B).

### 3.6 | FVIII half-life vs. plasma vWF concentrations

Linear regression of half-life on plasma vWF antigen concentrations shows that the half-life following administration of N8-GP tended to be longer in patients with higher baseline vWF antigen: slope estimate 12.58 (95% CI, 10.51-14.65) (Figure 3A). However, in children, there was less pronounced correlation between FVIII half-life and plasma vWF following administration of either N8-GP (slope estimate, 3.88 [95% CI, 0.87-6.88]) or the previous FVIII treatment (slope estimate, 1.78 [95% CI, 0.63-2.94]) (Figure 3B). Nevertheless, individual half-lives for N8-GP and previous FVIII product are presented in Figure 3C and show a consistently longer half-life for N8-GP compared to previous FVIII product for all patients at all levels of vWF.

**TABLE 3** Predicted steady-state PK of N8-GP and time with FVIII activity at >1 and >5 IU/dL

N8-GP dose	Dose interval (d)	Age (y)	Predicted parameters					
			Steady-state predose FVIII <sup>a</sup> (IU/dL) (ie, trough)	Steady-state postdose FVIII <sup>a</sup> (IU/dL) (ie, peak)	Time to FVIII 5 IU/dL (d)	% of week with FVIII > 5 IU/dL	Time to FVIII 1 IU/dL (d)	% of week with FVIII > 1 IU/dL
60 IU/kg	Q3D	0-11	2.8	112.4	2.5	84.6	3.8	100.0
	Q3/4D	0-11	2.8/0.8	110.4/112.3	2.5/2.5	72.3	3.8/3.8	97.9
50 IU/kg	Q3/4D	≥12	8.6/3.6	132.5/137.5	3.6/3.6	94.9	5.4/5.4	100.0
	Q4D	≥12	3.5	132.3	3.6	90.0	5.4	100.0

Notes: For all patients dosed at 60 IU/kg Q3D and Q3/4D, and at 50 IU/kg Q3/4D and Q4D, CL was predicted to be 2.8, 2.8, 1.5, and 1.5 mL/kg/h, respectively. In addition, for all patients dosed at 60 IU/kg Q3D and Q3/4D, and at 50 IU/kg Q3/4D and Q4D,  $V_{ss}$  was predicted to be 54.8, 54.8, 38.8, and 38.8 mL/kg, respectively. Predicted FVIII activity at SS was based on patient level data using a 1-compartment model with first-order elimination and PK parameter estimates (CL,  $V_{ss}$ ) obtained from single-dose 50 IU/kg PK parameters for each age group.

Abbreviations: CL, clearance; FVIII, factor VIII; PK, pharmacokinetic; Q3/4D, every 3/4 d; SS, steady state;  $V_{ss}$ , volume of distribution at steady state.

<sup>a</sup>The 2 values reflect that the peak and trough are not similar when the dosing intervals are not similar (ie, 3 and 4 d between dosing).

Higher vWF antigen levels were correlated with ABO blood type in adults as anticipated; patients with blood type O had lower vWF levels and also a lower half-life than non-O blood type adults (Table 4) or children (Table 5).<sup>22</sup>

## 4 | DISCUSSION

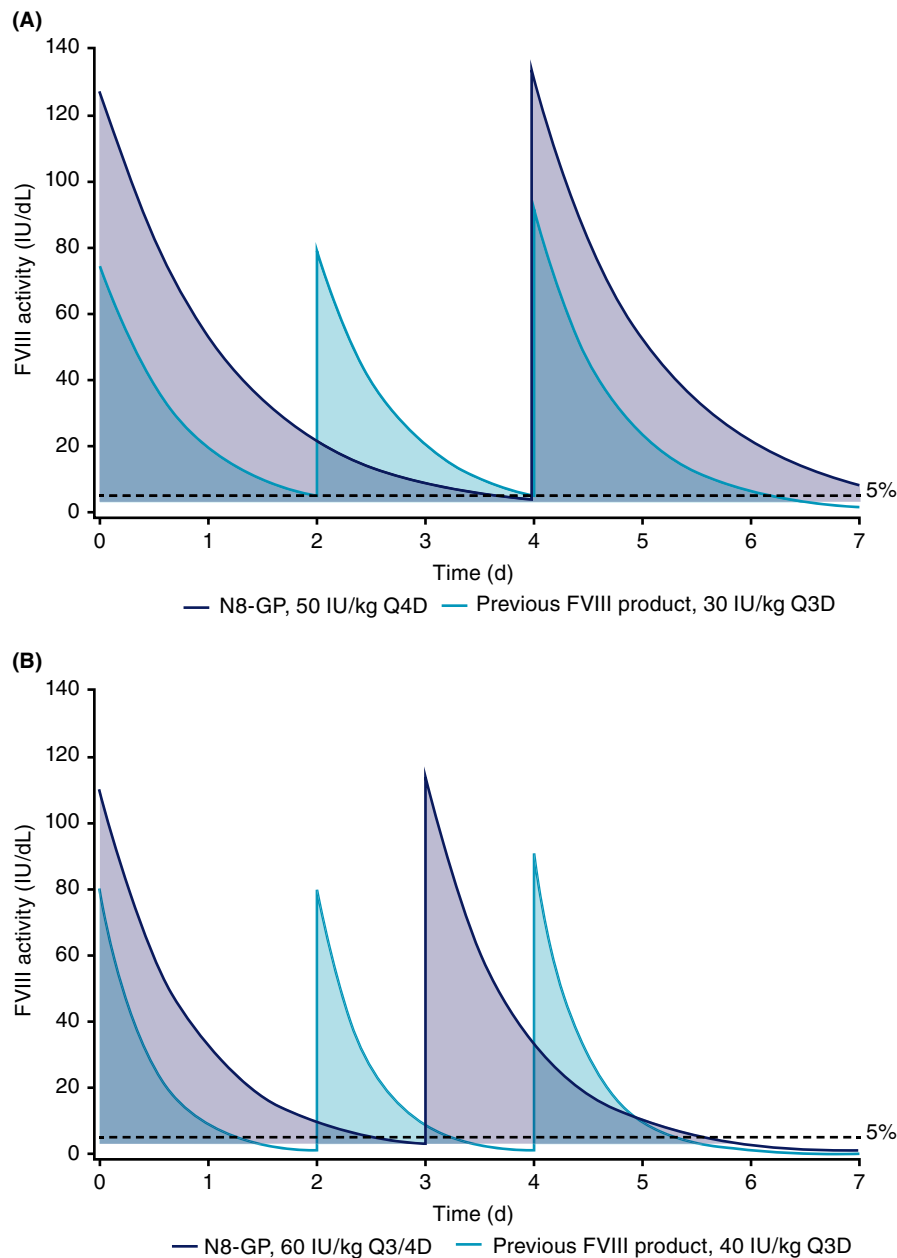
These analyses of data from clinical trials of N8-GP in children and adults/adolescents were conducted to characterize the PK profile of N8-GP. PK modeling predicted that children dosed every 3 days and adults/adolescents dosed every 3/4 days would maintain FVIII levels >5 IU/dL for >80% of the time, and >1 IU/dL for 100% of the time during prophylactic treatment with N8-GP. The association between half-life of N8-GP (ie, its stability) and endogenous vWF concentrations was also investigated.

This combined PK analysis shows that PK characteristics of N8-GP are consistent over time and supports the findings of pathfinder 1, in which N8-GP was shown to have dose-linear PK and a  $t_{1/2}$  1.6 times that of pdFVIII and rFVIII products in adults, and the corresponding prolongation of half-life of N8-GP compared to pdFVIII and rFVIII was 1.9 times in children (pathfinder 5).<sup>9,10</sup> Age-related differences in PK have also been reported for other rFVIII products<sup>23</sup> and attributed to a higher volume of distribution per kilogram of BW in children than in adults.<sup>24</sup> Theoretically, when dosing per kilogram of BW, patients with a high BMI will receive a higher absolute dose than patients with a low BMI. Assuming that plasma volume is subject to little change with increasing BMI, plasma FVIII activity would be expected to rise with increasing BMI. For N8-GP, this would correspond to rising IR and  $AUC_{0-inf}$  with increasing BMI and thereby a decrease in CL. However, only a limited effect of BMI on the PK parameters was observed (Table S1).

In recent years, a significant association between bleeding frequency and time spent with FVIII activity at <1 IU/dL has become evident, particularly in children. For example, in children aged 1 to

6 years, annualized bleed rate (ABR) has been shown to increase by 2.2% for every hour spent with activity <1 IU/dL.<sup>4</sup> Therefore, FVIII activity level of >1 IU/dL is an important threshold for the entire dosing interval during prophylaxis if breakthrough bleeding is to be minimized and outcomes improved.<sup>4,25</sup> PK predictions of N8-GP prophylaxis using a weight- and age-determined, fixed-dose regimen show that FVIII activity levels would be maintained within a range equivalent to mild-to-moderate hemophilia A (ie, >1 IU/dL) for the entire time (100%) between doses when administered Q4D in adults/adolescents, and for 94.9% of the time when administered Q3/4D in children. Although individualized prophylaxis based on each patient's individually determined PK is ideal,<sup>26</sup> in routine clinical practice this can be cumbersome and difficult to achieve. Therefore, there is increasing interest in the use of population PK to enable half-life prediction and dose calculation.<sup>27,28</sup> FVIII trough levels in the current study, both observed and predicted, demonstrate that a simple fixed interval and fixed weight-based dosing regimen can deliver the required minimum FVIII trough levels as a first step for successful prophylaxis in virtually all patients (adults/adolescents and children). Further clinical gains can be obtained by modifying the prophylaxis to patients' PK, clinical phenotype, joint status, and activity.<sup>29</sup>

Importantly, patients with severe hemophilia A of all ages treated with N8-GP achieved FVIII trough levels more than the suggested minimum with fewer injections, when compared to treatment with SHL FVIII products.<sup>8-10</sup> The higher FVIII trough levels with N8-GP give a better protective window in the event that patients miss/delay administering a prophylactic dose. The higher FVIII trough levels and better coverage with N8-GP can contribute to a reduction in bleed frequency and subsequent amelioration of target joint status. Like other EHL rFVIII products that provide sustained FVIII levels associated with mild-to-moderate hemophilia, N8-GP offers the benefit of reduced treatment burden.<sup>30,31</sup> This may lead to fewer acute bleeds, as adherence to the intended dose frequency is known to be a major determinant of overall treatment outcomes.<sup>4</sup>

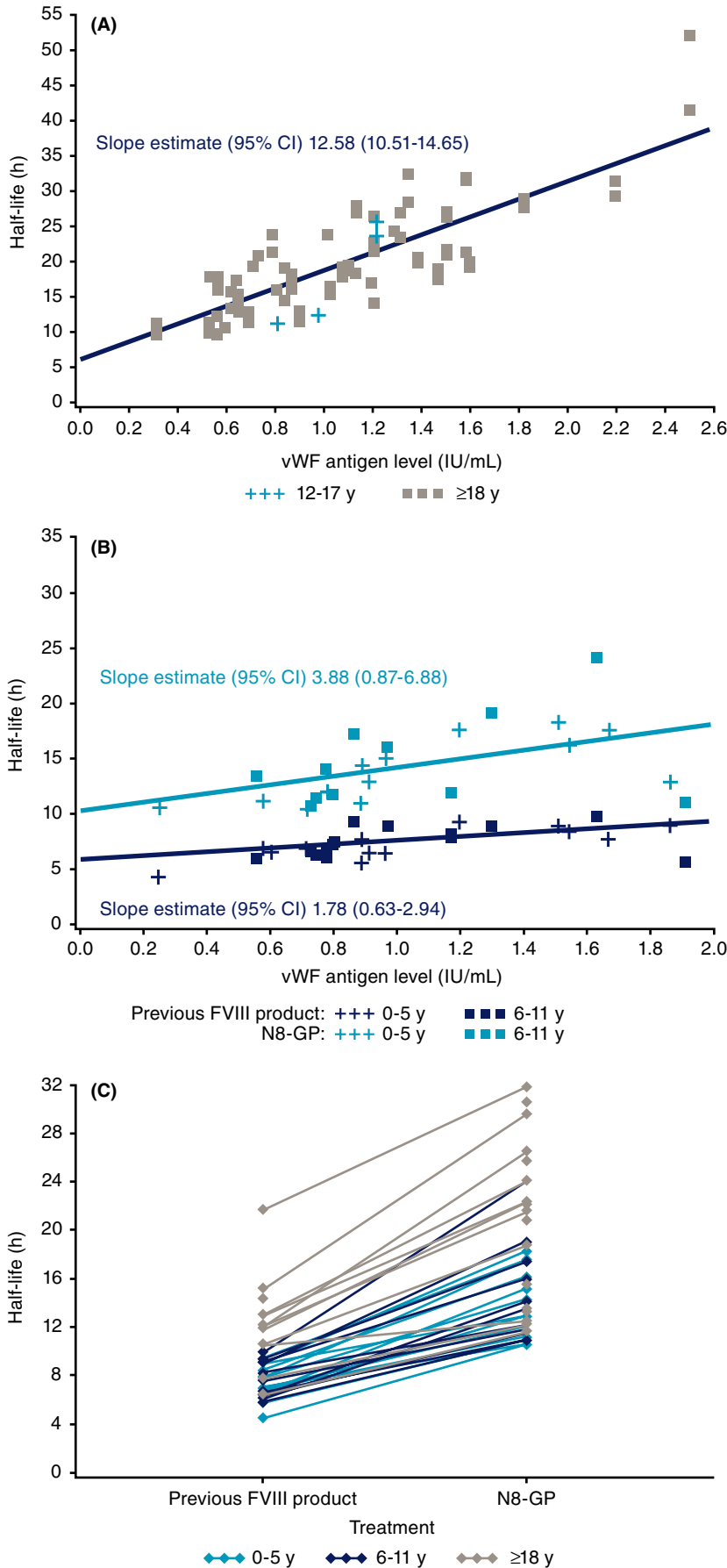


**FIGURE 2** Predicted FVIII activity profiles in patients with severe hemophilia A (A) aged  $\geq 12$  y, treated with N8-GP (50 IU/kg Q4D) or their previous FVIII product (30 IU/kg Q3D); and (B) aged  $< 12$  y, treated with N8-GP (60 IU/kg Q3/4D) or their previous FVIII product (40 IU/kg Q3D). The dashed horizontal line indicates 5 IU/dL activity (ie 5%). Shading denotes the time above critical FVIII activity level for N8-GP (gray) and previous FVIII product (blue). Predictions based on PK data obtained in pathfinder trials from: N8-GP pathfinder 1, 2, and 7; previous FVIII product: pathfinder 1 ( $\geq 18$  y). Previous FVIII was given 30 IU/kg Q3D (30 + 30 + 35 IU/kg) (2 + 2 + 3 d). Predictions based on PK data obtained in pathfinder 5 for both N8-GP and previous FVIII product. Previous FVIII product was given Q3D (40 + 40 + 45 IU/kg) (2 + 2 + 3 d). The patients' previous product could be either pd or rFVIII: 22 adults had rFVIII products (Advate, Kogenate, Kogenate FS, Helixate, Recombinate, ReFacto AF) and 4 had pdFVIII (Hemophil, Emoclot, Fanhdi) in pathfinder 2. In pathfinder 5, 22 children had rFVIII (Advate, Kogenate, Helixate FS, Recombinate, ReFacto AF, Xyntha, NovoEight), and 5 had pdFVIII (Immunate, Aleviate). Patients aged  $\geq 12$  y (A) dosed Q3/4D or Q4D with 50 IU/kg N8-GP were predicted to reach FVIII activity  $> 5$  IU/dL for 94.9% of the time and for 90.0% of the time, respectively, and were predicted to reach FVIII activity  $> 1$  IU/dL for 100% of the time with either dosing regimen. For patients aged  $< 12$  y (B) dosed with  $\sim 60$  IU/kg, FVIII activity of  $> 5$  IU/dL was also predicted for most of the interval with Q3/4D and Q3D (72.3% and 84.6%, respectively). Furthermore, FVIII activity  $> 1$  IU/dL was expected 97.9% of the time with the Q3/4D pediatric regimen and for the entire dosing interval for the Q3D. FVIII, factor VIII; pd, plasma-derived; PK, pharmacokinetic; Q3D, 3 times weekly; Q4D, every 4 d; Q3/4D, every 3/4 d; rFVIII, recombinant FVIII

The carrier molecule for FVIII is vWF, which is essential for maintaining FVIII stability and prolonging its half-life.<sup>12,32</sup> For rFVIII molecules to be effective for hemostasis, it is therefore important that

they interact with vWF as effectively as does native FVIII. Indeed, higher levels of endogenous vWF antigen have been shown to correlate significantly not only with a longer FVIII half-life, but also with





**FIGURE 3** Individual terminal half-life of FVIII activity following a dose of N8-GP vs. vWF antigen levels for (A) patients aged 12-17 and ≥18 y following a single dose of N8-GP 50 IU/kg in pathfinder 2 and 7 (n = 42 patients); and (B) patients aged 0-5 and 6-11 y following either a 50 IU/kg-dose of N8-GP or the patients' previous rFVIII product in pathfinder 5 (n = 24; FAS). (C) Individual terminal half-life for previous FVIII product and N8-GP for all patients (all age groups). CI, confidence interval; FAS, full analysis set; FVIII, factor VIII; rFVIII, recombinant FVIII; vWF, von Willebrand factor

**TABLE 4** Mean (range) half-life of N8-GP in adults ( $\geq 18$  y) according to blood type

Adults ( $\geq 18$ y)		
Blood type	Number of patients	Half-life (h)
A	11	23.7 (11.5-52.3)
AB	1	31.7 (31.7-31.7)
O	7	15.6 (9.8-21.5)
Missing	1	24.4 (22.5-26.3)

Note: Only patients who contributed to the primary PK analysis were included.

Abbreviations: PK, pharmacokinetic.

a lower ABR.<sup>13</sup> In the current analysis, positive linear correlations between plasma vWF and half-life were observed in patients who received N8-GP; this trend was less pronounced in children, but similar to their previous FVIII products. N8-GP has previously been shown to bind immobilized human vWF in vitro with similar affinity to native FVIII.<sup>14</sup> Furthermore, in mice lacking endogenous vWF, their half-life (with N8-GP) was shorter than in mice with endogenous vWF, but considerably longer than that of nonPEGylated FVIII in these animals.<sup>14</sup> With N8-GP, the half-life of drug not bound to vWF is extended by the polyethylene glycol moiety. The clinical implications of the relation between FVIII half-life and vWF may include the potential use of vWF antigen levels to help establish optimal EHL rFVIII dose regimens for patients with both low and high vWF levels, in conjunction with the monitoring of trough levels and bleed frequency.<sup>33</sup> However, further research is needed into the clinical utility of vWF levels in conjunction with the half-life of new EHL rFVIII products.

Several factors could have a role in the age differences observed in the correlations between FVIII and vWF half-life. The FVIII half-life is slightly shorter in children (13 hours in those aged 0-5 years

and 14 hours in those aged 6-11 years vs. 18 hours in patients aged  $\geq 12$  years), although still 1.9 times that of their previous rFVIII products.<sup>8,9</sup> vWF antigen levels can vary during childhood<sup>34</sup> and tend to increase as children get older.<sup>13</sup> However, these increases can be subtle and are not always detectable when vWF is measured at single time points (especially in children, in whom blood draws can be challenging<sup>6,35</sup>). Nevertheless, a consistently longer half-life for N8-GP compared to previous FVIII product for all patients was observed in the current study.

Potential limitations of the current analysis include the small number of patients undergoing PK assessment in pathfinder 2 and 5 (N = 24 and N = 27, respectively).<sup>8,9</sup> Additionally, the dosing regimen and PK prediction model assessed in this combined PK analysis were designed to evaluate the prevention of spontaneous bleeding only. In terms of analyzing the correlation between plasma vWF and FVIII activity half-life (and as mentioned above), single vWF measurements do not take into account the possibility of variation in vWF levels caused by physiologic stress.<sup>36</sup> Finally, discrepancies between 1-stage clotting and chromogenic assays have been documented with EHL rFVIII products.<sup>37,38</sup> PK assessments in the current study were based on FVIII activity in plasma as measured using a chromogenic assay. A recent field study showed that most clinical laboratories accurately measured N8-GP with either chromogenic assays or 1-stage clotting assays.<sup>39</sup> For the 1-stage clotting assay, some silica-based reagents should be avoided, as they cause underestimation.<sup>39</sup>

## 5 | CONCLUSIONS

The PK characteristics of N8-GP have been thoroughly studied and shown to be consistent over time. N8-GP prophylaxis with

**TABLE 5** Mean (range) half-life of N8-GP and patients' previous FVIII product in children ( $< 12$  y) according to blood type

Children ( $< 12$ y)			
Blood type	Patients in each group (n)	Half-life (h)	
		N8-GP (0-5 y) <sup>a</sup>	Previous FVIII product (0-5 y) <sup>b</sup>
O	N8-GP: 2 Previous FVIII product: 3	10.9 (10.6-11.2)	6.0 (4.5-6.9)
Non-O	7	15.2 (12.9-17.6)	8.0 (6.6-9.5)
N/A	4	13.0 (10.6-18.3)	7.2 (5.8-9.0)
Half-life (h)			
Blood type	Patients in each group (n)	Half-life (h)	
		N8-GP (6-11 y) <sup>a</sup>	Previous FVIII product (6-11 y) <sup>b</sup>
O	3	11.7 (11.0-12.2)	7.2 (5.8-8.2)
N/A	8	15.8 (10.9-24.0)	7.8 (6.1-9.9)

Notes: Only patients who contributed to the primary PK analysis were included.

Abbreviations: FVIII, factor VIII; N/A, not available; PK, pharmacokinetic.

<sup>a</sup>Assessed using a chromogenic assay with product-specific standard calibration.

<sup>b</sup>Assessed using a chromogenic assay with normal human plasma calibration.

predetermined fixed dose and interval achieved measured trough FVIII activity levels associated with moderate hemophilia for all ages: 1.2 and 3.0 IU/dL in children and adults/adolescents, respectively. Based on PK predictions of FVIII activity at steady state, adults/adolescents with severe hemophilia A treated prophylactically with N8-GP Q3/4D may be able to achieve FVIII levels associated with mild (>5 IU/dL) hemophilia 94.9% of the time and moderate (>1 IU/dL) hemophilia for the remaining 5.1% of the time; overall, no adults/adolescents had FVIII levels in the range of severe hemophilia at any point in time when dosed with N8-GP Q3/4D. We also observed that children had FVIII levels at or above the moderate hemophilia range 97.9% of the time when dosed Q3/4D. All of this comes with fewer injections compared with SHL rFVIII products. Additionally, as observed with SHL rFVIII and endogenous FVIII, linear correlations between vWF and N8-GP half-life were observed, with differences between adults/adolescents and children. We recommend fixed N8-GP dosing regimens for patients with severe hemophilia A in the first instance to achieve adequate coverage during the dosing interval, with subsequent modification based on clinical response, trough levels, or patient-specific PK.

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PC has received grants/research support from Bayer, CSL Behring, Novo Nordisk, Pfizer, and SOBI AB; and reimbursement for consulting from Baxalta (Shire), Bayer, Baxter, Bioverativ (Biogen), CSL Behring, Freeline, Novo Nordisk, Pfizer, Roche, Shire, and SOBI. MC has received grant/research support from Baxalta (Shire), Bayer, Bioverativ, Novo Nordisk, and Pfizer; reimbursement for consulting from Baxalta (Shire), Bioverativ, CSL Behring, Novo Nordisk, Octapharma, and Pfizer; and speaking for Baxalta (Shire), Bayer, Bioverativ, CSL Behring, Novo Nordisk, Octapharma, and Pfizer. PAH has received reimbursement for research support from Bayer, Octapharma, Pfizer, and Shire; and consulting from Bayer, CSL Behring, Novo Nordisk, Pfizer, Roche, Shire, and SOBI. VJ-Y has received reimbursement for attending symposia/congresses and/or honoraria for speaking and/or honoraria for consulting, and/or funds for research from Bayer, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Shire, and SOBI. SRLentz has received grant support and personal fees from Novo Nordisk A/S and has

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## AUTHOR CONTRIBUTIONS

PC, MC, PAH, VJ-Y, SRL, LHP, AT, AW, ES were the clinical investigators during the trials; JM and CS undertook the statistical analysis and analyzed the data. All authors directed the data analysis and the development of the manuscript and approved the final version of the manuscript.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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