Elucidation of Factor VIII Activity Pharmacokinetics: a Pooled Population Analysis in Hemophilia A Patients Treated with Moroctocog Alfa

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Abstract

This study investigated the disposition of coagulation factor VIII activity in 754 moderate to severe hemophilia A patients following the administration of moroctocog alfa, a B-domain deleted recombinant factor VIII. Data analyzed included patients aged 1 day to 73 years enrolled in 13 studies conducted over a period of 20 years in 25 countries. A two-compartment population pharmacokinetic model with a baseline model described the pooled data well. Body size, age, inhibitors, race and analytical assay were identified as significant predictors of factor VIII disposition. In addition, simulations of prophylactic dosing schedules in several pediatric cohorts showed large variability and suggest that younger patients would require higher weight-adjusted doses than adolescents to achieve target factor VIII trough activity, when receiving every other day or twice weekly dosing.

Introduction

Hemophilia A is a hereditary bleeding disorder caused by a qualitative or quantitative deficiency of coagulation factor VIII (FVIII), with a prevalence of 1 in 5,000 male births (1). The characteristic phenotype in hemophilia A is the bleeding manifestations that affect soft tissues, joints and muscles and debilitating chronic arthropathy in the long term (1). The severity of the disease depends on the amount of endogenous functional FVIII an individual is able to synthesize and is classified as severe (<1 IU/dL [<1% of normal] endogenous FVIII activity), moderately severe (1-2 IU/dL), moderate (2-5 IU/dL) and mild (5-40 IU/dL) (2).

The main treatment of hemophilia A is replacement therapy which relies on the administration of exogenous FVIII to reach adequate hemostasis. On-demand treatment is given at the time of bleeding and the doses, frequency and duration of treatment depend on the type and severity of the bleeding event (2). Prophylactic therapy aims at preserving the normal musculoskeletal function, and is usually given as 25-40 IU/kg (Malmö protocol) or 15-30 IU/kg (Utrecht protocol) per dose administered three times a week (2, 3).

The pharmacokinetic (PK) characterization as well as potency of marketed products are based on measurements of FVIII activity and are expressed in international units (IU), where one IU of FVIII approximates the activity of FVIII in 1 mL of normal human plasma. The one-stage activated partial thromboplastin time clotting assay (OSA) and the chromogenic substrate assay (CSA) are the most commonly used assays for FVIII activity measurement. Specific phenotypes, mutations in F8 gene and type of FVIII product have been found to lead to discrepant results between these assays (4),

being particularly large for B-domain deleted recombinant FVIII (BDDrFVIII) products with lower activity measured with the OSA compared with CSA (4, 5).

Moroctocog alfa is a BDDrFVIII product indicated for use in adults and children with hemophilia A for control and prophylaxis of bleeding episodes and perioperative management. The same moroctocog alfa product is marketed under two trade names, Xyntha® and Refacto AF® (Wyeth Pharmaceuticals Inc. [Pfizer], Philadelphia, USA), with potency assigned using the OSA (USA, Canada and other regions) or the CSA (European Union and other regions), respectively. These products were preceded by Refacto® (Wyeth Pharmaceuticals Inc. [Pfizer], Philadelphia, USA). There is limited information on population PK of FVIII activity following administration of any of the BDDrFVIII products, but both single and biphasic disposition characteristics have been suggested (6, 7). Compared to adults, pediatric patients are usually found to have a lower recovery (i.e. peak FVIII activity in IU/dL divided by dose in IU/kg) following the administration of FVIII products, a higher body weight-adjusted clearance (CL), and a shorter terminal elimination half-life ($t_{1/2}$) (8, 9).

The aims of this study were to 1) characterize the population PK of FVIII activity following treatment with moroctocog alfa in moderate to severe hemophilia A patients employing all available data from clinical development trials, 2) identify potentially relevant covariate relationships and 3) simulate trough FVIII activity values to assess dosing schedules for prophylaxis in pediatric cohorts (0 - <2 years, 2 - <6 years, 6 - <12 years and 12 - <17 years).

Results

Data

A total of 754 moderate to severe hemophilia A patients were included in the analysis and 234 patients (31% of total) were younger than 17 years at trial start. The demographic characteristics are provided in **Table 1**. The median patient was a 23-year-old non-Hispanic/Latino white male, weighing 69 kg.

A total of 7,363 FVIII activity observations were used to develop the model, whereof 910 (12.4%) were below the lower limit of quantification (LLOQ) (**Figure 1**). For 312 sampling times (4.2%), 2 or more measurements were reported. The median dose was 2,000 IU (range 100-40,000), corresponding to 32 IU/kg (range 2-1,300 IU/kg).

Population PK model

The PK of FVIII activity was best described by a two-compartment model with linear first-order elimination, parameterized as CL, inter-compartmental clearance (Q), central (V_1) and peripheral volume (V_2). Covariate relationships related to body size, age, inhibitors, race and assay were identified.

The final parameter estimates and respective equations are provided in **Table 2.** Population prediction corrected visual predictive checks (pcVPCs) (**Figure 2**) and other goodness-of-fit plots (**Supplemental Figure S1**) show no marked bias, substantiating that the general trend in the data and the variability is well captured, with only some instances of a slightly over-predicted variability at a study level. The over-prediction of some FVIII activities below the LLOQ, may be due to inaccurate dosing or sampling times.

A three-compartment model was not statistically superior to a two-compartment model (P=0.047, 2 degrees of freedom, *df*) which in turn was statistically superior to a one-compartment model (P<0.0001, 2 *df*). The baseline model describes the individual

predictions of FVIII ($FVIII_i$) as the sum of the endogenous (hemophilia A severity), exogenous (FVIII administered) and potential residual FVIII activity (a FVIII activity before the first dose higher than disease severity activity allowed in each clinical trial), the latter decaying according to the individual terminal phase in the model (graphical illustration in **Supplemental Text S1**):

$$FVIII_i = \begin{cases} Endogenous_i + Exogenous_i & ;if\ first\ pre-dose\ FVIII\ observation \leq degree\ of\ severity\ activity \\ Endogenous_i + Exogenous_i + Residual_i & ;if\ first\ pre-dose\ FVIII\ observation > degree\ of\ severity\ activity \end{cases}$$

The endogenous FVIII activity was bimodally distributed and a mixture model, estimating the probability of patients to have one of two levels of disease severity, was statistically significant (*P*<0.0001, 2 *df*; inter-individual variability (IIV) decreased tenfold). The inclusion of inter-occasion variability (IOV) on CL and V_2 significantly improved the model (*P*<0.0001, 2 *df*) and decreased the residual error magnitude as well as IIV on CL and V_2. A proportional residual error model with a component accounting for correlated residual error for replicate measurements (*P*<0.0001, 1 *df*) was applied.

The inclusion of total body water on disposition parameters with theory-based allometric exponents resulted in a better fit compared to corresponding models with weight (Δobjective function value (OFV)=+38.5), lean body weight (ΔOFV=+50.4), or no body size measure (ΔOFV=+1,141). However, weight was the preferred measure of body size given that the two measures were strongly correlated (r^2 of 0.963) and the predictions between the models did not differ substantially.

The effect of analytical method (OSA or CSA), inhibitor status (positive or negative) and age, all covariates expected to influence the FVIII activity, were

statistically significant. The FVIII activity as measured by the CSA was estimated to be systematically higher than when measured with the OSA for central and local laboratories (*P*<0.0001, 2 *df*). Also, higher residual error magnitude was estimated when the OSA was applied (*P*<0.0001, 1 *df*). A higher CL was estimated for inhibitor positive subjects (dichotomous inhibitor status, P<0.0001, 1 *df*). Age influenced CL in a non-linear fashion (*P*<0.0001, 2 *df*), predicting an increase in CL up to one year of age and thereafter a decrease in CL according to:

$$\text{Typical CL (dL/h)} = 2.76 \times \left(\frac{WTi}{70}\right)^{3/4} \times \text{age effect} \times (1 + 1.66 \times \text{INH}_i) \times (1 - 0.347 \times \text{STUD})$$

$$\text{age effect} = \begin{cases} 1.13 + 0.149 \times (\text{AGE}_i - 1) & ; if\ age \leq 1\ year\ old \\ 1 - 0.00678 \times (\text{AGE}_i - 20) & ; if\ age > 1\ year\ old \end{cases}$$

where WT_i is body weight in kg, AGE_i is age in years, INH_i is inhibitor status (1 positive, else 0), STUD is study effect (1 for study B1831090, else 0). Body weight and age were centered at 70 kg and 20 years old, for ease of interpretation.

Exploration of additional covariates by stepwise covariate model building (SCM) and/or graphical exploration, revealed subjects of black race to have a higher V_2 compared with other races (*P*<0.0001, 1 *df*) and a study-specific effect on CL (*P*=0.0010, 1 *df*). All covariate relationships remained statistically significant in a backwards deletion procedure and none was classified as unlikely to be clinical relevant.

Illustration of covariate relationships

Deterministic simulations illustrating the consequences of the identified covariate effects on the FVIII activity-time profiles (**Figure 3A)** clearly show the major impact of inhibitor occurrence, resulting in a reduction of 5.28 h on the terminal $t_{1/2}$ and a 62.3% lower overall exposure compared with not having inhibitors and falling outside the 95%

prediction interval (PI) of FVIII activity for a typical individual taking IIV and IOV into account. Also, the small consequences of the other covariates are contrasted, with black subjects having a 26% longer terminal $t_{1/2}$ than other races and 57-year-old (high age) subjects having a 33% longer terminal $t_{1/2}$ than 20-year-old subjects, all within the 95% PI. Furthermore, the FVIII activity-time profiles following administration of 50 IU/kg of Xyntha in subjects with varying age and body weight (**Figures 3B),** and the relationships of CL and terminal $t_{1/2}$ with weight/age (**Figures 3C and 3D)** demonstrate that employing a body weight adjusted dose is not sufficient if aiming for similar exposure in adults and younger patients.

Simulations

The simulated individual trough FVIII activity following the first and last dose during week 3, across a range of doses and pediatric age cohorts, are given in **Figure 4A**. Following the once weekly schedule, the 95% prediction intervals of the observed trough FVIII activity are below 1 IU/dL across all age cohorts and doses. Following twice weekly dosing, the median trough activity is approaching 1 IU/dL at doses above 60 IU/kg for the cohort 12 - <17 years only, but for the remainder, the trough activity on the median is predicted markedly below 1 IU/dL. To reach median activities of 1 IU/dL dosing every other day appears to be required. This every other day schedule results in a minimum of 20% of the subjects not reaching activities above 1 IU/dL at trough following the highest dose (100 IU/kg) for adolescents and 40% for the youngest children (**Figure 4B**).

Discussion

This population PK study is currently the largest to report the PK parameters for FVIII activity following administration of any rFVIII product in moderate to severe hemophilia A patients across a broad age spectrum, size range, and ethnicity/race diversity. The final model is a two-compartment model with linear first-order elimination, combined with a baseline model and includes parameter-covariate relationships related to body size, age, inhibitors, race and assay.

The estimated values of CL (2.76 dL/h) and steady-state volume of distribution (3.38 L) are similar to those previously reported for moroctocog alfa (7) and other rFVIII products (7-12). Furthermore, the model-predicted recovery for a typical patient as measured by CSA, calculated as ($FVIII_{30\text{-}min}$ – $FVIII_{pre\text{-}dose}$) / (dose/body weight), was 2.10 IU/dL/IU/kg, confirming that each unit infused per kg of body weight increases plasma FVIII activity by 2 IU/dL (13).

The baseline model identified two sub-populations with endogenous activity corresponding to disease status of severe and moderately severe hemophilia A, at 0.47 IU/dL and 1.59 IU/dL, respectively. Furthermore, residual FVIII activity, due to for instance an incomplete wash-out, was described in the baseline model. Instead of subtracting a pre-dose activity from subsequent measurements, we accounted for the decrease of high pre-dose activity as well as for endogenous activity. This method leads to more accurate parameter estimates for the exogenous FVIII activity, based exclusively on the activity through the dose administered.

Most of the subjects included in this study had FVIII measurements on more than one occasion with more than one sample on at least one occasion. The magnitude of IOV, 34.7% for CL and 41.0% for V_2, was high, contrasting previous reports of low or non-significant IOV (8, 9). Possible causes for this finding are the pooled nature of this

analysis, including data from studies with very different designs and variable times between occasions (from a few days up to several months). Further, the IOV may reflect unknown variations in covariates over time (e.g. inhibitor titer, von Willebrand factor [vWF]). The fact that the IOV on the PK parameters was larger than the IIV, limits the applicability of therapeutic drug monitoring for dose individualization. The consequences of this finding for dose individualization require further investigation.

Inhibitors (circulating neutralizing antibodies against FVIII) are known to increase the CL of FVIII, sometimes requiring massive doses or the use of bypassing agents to control bleeding episodes. The prevalence among patients with severe hemophilia A is 12-13% and the incidence approximately 30% (14). In our pooled data the occurrence of inhibitors was low (5.97%), probably related to that most patients were previously treated patients and patients with history of inhibitors were not usually eligible for enrollment into the clinical studies (1). Studies characterizing the relationship between CL of FVIII and inhibitor titers in a clinical context are lacking (15). In this study, the dichotomous inhibitor status was found to be more informative than the continuous inhibitor titers, possibly because the analytical method to measure the inhibitor varied between studies, and/or the presence of different types of inhibitors with different kinetics (14, 16). The occurrence of inhibitors (>0.6 Bethesda units/mL, in which 92.5% were low titers [<5 Bethesda units/mL]) was related to a more than doubling in CL. From all identified covariates, this was the factor that had the most profound effect on the PK of FVIII activity, indeed impacting treatment.

The total volume of distribution (3.4 L) implies a distribution of FVIII mainly in plasma. Several reasons have been proposed to justify the identification of a two-compartment structure including adsorption to vessel walls, a rapid elimination process

(17) or the binding of FVIII to vWF (18). The finding that black subjects had an 88% higher V_2 compared with other races (1.7 L versus 0.92 L) was unexpected, potentially explained by differences in binding of FVIII to vWF, since black subjects have been shown to have higher levels of vWF than white subjects (19, 20). The precision of the estimated effect was low (95% CI 37-151%), not unexpected given the small number of black subjects (N=10). Thus, the identified effect should be interpreted with caution. Furthermore, the clinical consequence of the relationship found with initially lower FVIII followed by a somewhat longer terminal $t_{1/2}$ activity in black subjects as compared with non-black subjects (**Figure 3A**), is considered limited.

The discrepancy of measurements of FVIII activity between the OSA and the CSA (-39%) is in line with previous studies (4, 5, 18). The lower discrepancy for one local laboratory (-15%) can be justified by the high inter-laboratory variability recognized for the OSA (4). We assumed the same relative difference between the methods irrespective of activity, supported by a previous report that did not identify a difference in the terminal $t_{1/2}$ based on samples assayed with CSA or OSA (7).

PK studies involving moroctocog alfa administration to a large number of pediatric patients are scarce. The very young children in this study were usually assessed with only peak and trough measures of FVIII activity. A nonlinear relationship between CL and age was identified, where body weight-adjusted CL increased over the first year of life, and thereafter decreased (**Figure 3C**). Although the relationship carries high imprecision for the low age group (up to 1 year), the 95% CI for the estimated covariate effect did not include 0 (95% CI 0.0116-0.352). This study confirms the relationship between CL and age described in the literature for FVIII activity (8, 9) and adds information for the age range 0-1 years old. The effect of age markedly affects the

disposition of FVIII, particularly in young children with a need of higher doses per kg of body weight. The mechanism behind the age effect is not known. However, it may, at least in part, be explained by differences in the vWF levels, since low vWF levels are known to lead to decreased FVIII activity (i.e. increased CL) (21), and vWF levels have been described to vary with age (22, 23). High vWF levels were observed at birth, decreasing over the first year of life, and thereafter gradually increasing during childhood (22), thus in agreement with our findings. However, further studies are required for firm conclusions.

The simulated FVIII activity for the pediatric age cohorts exhibited large variability, a feature mainly governed by the relatively large IOV for CL and V_2. The simulations revealed that dosing once weekly would be unlikely to result in FVIII activity above 1 IU/dL over much of the dosing interval, and to reach this treatment target would require dosing twice weekly or every other day using high weight-adjusted doses. The fact that younger patients need higher doses per kg body weight than the typical adult patient to reach the same trough activity is not new, but has now been characterized in this large data set, which included children between 0-1 years using population modeling. Collins *et al.* found that to maintain a factor activity above 1 IU/dL in 1-6 years old children following a Monday/Wednesday/Friday dosing would require a median dose per week of 132 IU/kg and a dose on Friday that varied between 22 IU/kg and 410 IU/kg (24). A survey of prophylaxis in Canada (25), revealed three times weekly as the most common regimen (40% of the patients) followed by twice weekly in 25%, while weekly, alternate days and daily was less commonly applied. The variability seen in those common regimens may have translated from the variability between patients in their PK, bleeding phenotype or physical activity level. Further investigations are warranted to find an

optimal dosing schedule in pediatrics taking into account the trough FVIII activity as well as other important factors.

A study effect was identified on CL and the typical PK parameters describe the FVIII activity for the remaining 12 studies, which was supported by diagnostics suggesting that the study effect on CL was sufficient to avoid bias in the overall fit. Accordingly, the inclusion of the study effect allows the model to be used in a prospective manner.

The analyzed data exhibited varying features with respect to study design (aim and duration, doses and FVIII sampling strategy) and patient characteristics which involve difficulties in model identification given the sparseness of data in certain circumstances. Some characteristics that previously have been associated with FVIII activity PK, for example blood group and vWF (21, 23, 26, 27) were not available, which is a limitation of this study.

In summary, the developed population PK model of FVIII activity based on a large heterogeneous population of moderate to severe hemophilia A patients, and subsequent simulations, confirm and extend the current knowledge about FVIII activity PK. The estimated population PK parameters, combined with complete dosing information and important patient characteristics can serve as an aid to individualize dose regimens, with the ultimate goal of safe and effective treatment with moroctocog alfa. In addition, the developed model can be used to explore the link between FVIII plasma activity and the risk of bleeding, and thereby aid in improving therapy aiming to protect more effectively against joint and non-joint bleeding due to hemophilia A.

Methods

Studies and patients

The study included hemophilia A patients enrolled in 13 clinical trials involving intravenous administration of moroctocog alfa (ReFacto, ReFacto AF or Xyntha) and with at least one plasma FVIII activity measured. The trials were conducted between 1993 and 2013 in 25 countries, including both pediatric and adult patients, whereof 91 were previously untreated patients. Informed consent was obtained from all subjects and the study protocols were approved by each participating center's ethics committee or institutional review boards. The studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. An overview is available in **Table 3** (6, 28-35) and details on unpublished studies are available in **Supplemental Text S1**.

Sampling and measurement of FVIII activity

Four studies aiming at evaluating PK involved single intravenous dosing followed by frequent sampling up to 48 or 72 h post-dose. Four efficacy, safety and/or PK studies included frequent sampling on at least one occasion and sparse sampling on one or more occasions. Five remaining studies, evaluating safety and/or efficacy, involved 2-4 samples per occasion. FVIII activity was measured with the OSA or CSA at a central (12 studies) or local laboratory (study B1831003) with an LLOQ of 1 IU/dL (2 IU/dL in two studies).

Data analysis

The FVIII activity time course was analyzed with a population approach using non-linear mixed-effects modeling (36). The model was developed using NONMEM (version 7.3; Icon Development Solutions, Hanover, MD) with the first-order conditional estimation

algorithm with eta-epsilon interaction (37). Model diagnostics were supported by Perl-speaks-NONMEM (PsN, version 4.6.0) and Xpose (version 4.5.3) (38) and data checkout and management, statistical summaries and model diagnostics were carried out in R (version 3.2.2). Model selection was based on the OFV, goodness-of-fit plots, precision in parameter estimates and scientific plausibility. A competing hierarchical model was selected if the OFV (approximately χ^2 distributed) was reduced by at least 6.63, corresponding to a statistical significance level of $P \leq 0.01$ (1 *df*). Data below the LLOQ were used when the actual values were available or otherwise replaced by LLOQ/2 (39). Missing covariate values were imputed by carrying backward and/or forward available values (body weight, inhibitor status), replacement by the most common category (ethnicity) or by regression analysis (total body water, lean body weight). Missing height information, required to calculate total body water and lean body weight, was imputed based on the height-weight relationship estimated from the data of the remaining patients:

$$HT_i = 51.6 + ((136 * WT_i^{2.04}) / (22.4^{2.04} + WT_i^{2.04})) + \eta_i$$

where HT_i represents the height (cm) for a given subject with weight WT_i (kg) and an individual variability parameter $\eta_i \sim N(0, 47.8)$ (SD = 6.91 cm).

Model development: structural and stochastic model

A two-compartment model with first-order elimination was used as a starting point and alternative disposition models were investigated. The difference in potency between products was accounted for by setting the bioavailability term in NONMEM (F) of Xyntha (OSA calibrated) to 1.38 relative to ReFacto products (CSA calibrated) (13).

Since the data included patients with various levels of hemophilia A severity as well as residual amounts of FVIII activity at the start of the study, a baseline model was developed.

IIV and IOV were described assuming log-normal distributions of the structural model parameters (40). An occasion was defined as each separate dose event with associated measurements of FVIII activity (pre- or post-dose). Residual variability was parameterized using an additive, proportional or combined additive and proportional model. Replicate residual variability was taken into account for repeated measurements of FVIII activity from the same sample (41). Equations used to develop the model are described in **Supplemental Text S1**.

Model development: covariate model

Initially, the influence of body size on the disposition parameters was explored using fixed allometric theory-based exponents (¾ and 1 for clearances and volumes, respectively) or estimating allometric exponents (42). The tested measures of body size were weight, lean body weight (James formula), and total body water (Mellits-Cheek formula age <18 years or Watson formula age ≥18 years) (43-45). Thereafter, covariates expected to influence the FVIII activity were explored on some selected parameters: analytical method on F and on residual error magnitude, inhibitor status and titer on CL, and age on CL. Additional relationships (age, race and ethnicity) were assessed through the SCM procedure in PsN, using significance levels of $P \leq 0.01$ and $P \leq 0.005$ in the forward and backward step, respectively (46). Study, year of study start and country were explored graphically and further investigated within the model if necessary. The final inclusion was based on both statistical significance (final backward deletion

procedure) and unlikely clinical relevancy. A dichotomous covariate was judged as unlikely clinically relevant if the change in the typical parameter value was <10% from the most common category, and a continuous covariate if the difference from the typical parameter value at the 50th percentile of the covariate distribution was <5% at the 5th and 95th percentiles of the covariate distribution.

Model qualification

Key-models were qualified using pcVPCs (200 replications) (47). The reliability of the individual PK parameters and diagnostic plots was judged based on the magnitude of eta- and epsilon-shrinkage (48). The precision of parameter estimates was obtained from the covariance step in NONMEM. For the final model, standard errors and confidence intervals were estimated using the sampling importance resampling (SIR) procedure (49).

Monte Carlo simulations

The final population PK model was used to simulate individual trough FVIII activity (i.e. residual error variability not included) for the 4 cohorts of pediatric patients (0 - <2 years, 2 - <6 years, 6 - <12 years and 12 - <17 years) following administration of doses of Xyntha ranging between 10-100 IU/kg administered once weekly, twice weekly and every other day, up to 3 weeks of treatment. For each cohort body weight was sampled from the National Health and Nutrition Examination Survey (NHANES) data from years 1999-2010, publicly available at Centers for Disease Control and Prevention (CDC) (50). Each cohort included approximately 1,000 individuals uniformly sampled over the age range on a month resolution level.

Additional Supporting Information can be found in the online version of this article.

Study Highlights

- **What is the current knowledge on the topic?**

Hemophilia A has an overall low prevalence and pharmacokinetic (PK) studies with factor VIII (FVIII) replacement products usually involve small groups of patients. A study reporting the PK in a large population of heterogeneous patients is missing.

- **What question did this study address?**

This study characterizes the population PK of FVIII activity following administration of moroctocog alfa in 754 hemophilia A patients. In addition, dosing schedules for prophylaxis were assessed in several pediatric cohorts through simulations.

- **What this study adds to our knowledge**

Body size, age, inhibitor status, race and assay were identified as predictors of FVIII disposition. Younger patients require more often, higher weight-adjusted doses than adolescents to achieve the same FVIII trough activity. Quantification of the effect of low titer inhibitors has not been reported previously and the finding of the impact of race suggests further exploration.

- **How this might change clinical pharmacology or translational science**

The presented findings extend the current knowledge about FVIII activity PK in humans and contribute to a more personalized treatment of hemophilia A patients.

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Conflict of Interest/Disclosure

This study was sponsored by Pfizer Inc. J.A.A., E.I.N. and S.J. are employees of the Department of Pharmaceutical Biosciences, Uppsala University. J.K.B. and L.H. are employees of Pfizer Inc.

Author Contributions

S.J., J.A.A., E.I.N., J.K-B., and L.H. wrote the manuscript; S.J., J.A.A., E.I.N., J.K-B., and L.H. designed the research; S.J., J.A.A., E.I.N., J.K-B., and L.H. performed the research; S.J., J.A.A., E.I.N., J.K-B., and L.H. analyzed the data.

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Table titles

Table 1. Summary of baseline demographic data and occurrence of inhibitors for all included patients

Table 2. Final model parameter estimates for factor VIII activity following administration of moroctocog alfa measured with the chromogenic substrate assay

Table 3. Overview of clinical trials included in the population PK analysis

Figure legends (color version)

Figure 1. Observed post-dose factor VIII activity (non-adjusted for pre-dose activity) and percent of observations below the lower limit of quantification (LLOQ) *versus* time after start of infusion. Observations below LLOQ are represented as LLOQ/2 if a measured activity was not available.

Figure 2. Population prediction-corrected visual predictive checks by study for the final model. The y-axis is logged to emphasize later times after start of infusion. Dots represent the individual predicted-corrected factor VIII activity observations, solid and dashed red lines represent the median and the 2.5th and 97.5th percentiles of the observed data, respectively, and shaded areas the simulation-based 95% confidence interval for the corresponding percentiles.

Figure 3. Top panels illustrate the effect of covariates on the population predictions of FVIII activity versus time for severe hemophilia A subjects, with varying characteristics following the administration of 3,500 IU of Xyntha overlaid with the 95% prediction interval of FVIII activity for the typical subject, taking inter-individual and inter-occasion variability into account (A) and with varying age and weight following the administration of 50 IU/kg of Xyntha (B). The typical subject is a 20-year male weighing 70 kg, being non-Hispanic/Latino, non-black race, inhibitor negative and not belonging to study B1831090. High age (57 years old) and high weight (105 kg) correspond to the 97.5th percentile of the observed data. The lower panels show the typical clearance normalized by body weight (clearance per kg) *versus* age (C) and the typical terminal half-life *versus* age (D). CSA, chromogenic substrate assay.

Figure 4. Simulations of trough FVIII activity following three dosing schedules (once weekly, twice weekly and every other day over 3 weeks) in four pediatric cohorts across a wide range of doses: (A) trough FVIII activity following the first (twice weekly schedule only) and last dose in week 3 and (B) percentage of patients having a trough factor VIII activity <1 IU/dL, following the first (twice weekly schedule only) and last dose in week 3. In panel (A) median trough FVIII activity values above 1 IU/dL are represented in blue. PI, prediction interval.

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Supplemental file titles

Supplemental Text S1: Additional details on results and methods

Supplemental Figure S1: Additional prediction- and simulation-based goodness-of-fit plots for the final population pharmacokinetic model

Table 1. Summary of baseline demographic data and occurrence of inhibitors for all included patients

Characteristics	Value	Missing, N (%)
Age at study entry (years), median (range)	23 (0.0027-73)	0 (0)
Weight (kg), median (range)	-	-
0- <1 year, N=62	8 (3-12)	3 (0.4)
1- <2 years, N=21	11 (9-14)	0 (0)
2- <6 years, N=8	17 (11-20)	0 (0)
6- <12 years, N=25[a]	30 (21-57)	0 (0)
12- <17 years, N=118	56 (34-109)	0 (0)
All data, N=754	69 (3.0-134)	4 (0.5)
Lean body weight (kg), median (range)	53 (2.8-78)	283 (38)
Total body water (L), median (range)	40 (1.7-63)	283 (38)
Sex	-	0 (0)
Male, N (%)	753 (99.9)	-
Female, N (%)	1[b] (0.1)	-
Race	-	0 (0)
Asian, N (%)	58 (7.7)	-
Black, N (%)	10 (1.3)	-
Other, N (%)	29 (3.8)	-
White, N (%)	657 (87)	-
Ethnicity	-	19 (2.5)
Hispanic/Latino, N (%)	39 (5.2)	-
Not Hispanic/Latino, N (%)	696 (92)	-
Inhibitor status[c]	-	1,051 (14) [n]
Negative, n (%)	6,138 (83)	-
Positive, n (%)	174 (2.4)	-

N, number of subjects; n, number of observations
[a]Two patients belonging to cohorts 6-<12 years and 12-<17 in different studies were only counted once in this summary
[b]Homozygous female with hemophilia A
[c]Positive if >0.6 Bethesda units/mL

Table 2 Final model parameter estimates for factor VIII activity following administration of moroctocog alfa measured with the chromogenic substrate assay

Parameter	Estimate	SIR %RSE[a]	SIR 95% CI[a]	
			lower	upper
Structural model				
CL[b], dL/h	2.76	2.13	2.65	2.88
V_1[c], L	2.45	2.24	2.35	2.57
Q[d], dL/h	25.1	12.2	19.7	31.8
V_2[e], L	0.923	5.92	0.833	1.04
Endogenous FVIII activity 1, IU/dL	0.474	0.641	0.468	0.479
Endogenous FVIII activity 2, IU/dL	1.59	1.95	1.53	1.65
Fraction of patients belonging to subpopulation endogenous FVIII activity 1	0.803	2.11	0.768	0.837
Fraction of patients belonging to subpopulation endogenous FVIII activity 1 within studies B1831015 and B1831053	0.110	22.5	0.0679	0.164
Residual activity[f], IU/dL	2.84	8.58	2.40	3.37
Covariate model				
Inhibitor status on CL[b], % difference if positive	166	9.72	125	188
Age on CL up to 1 year old[b]	0.149	60.1	0.0116	0.352
Age on CL above 1 year old[b]	-0.00678	12.2	-0.00854	-0.00526
Study effect on CL, % difference if study B1831090[b]	-34.7	12.6	-40.7	-24.2
Allometric exponent for V_1 and V_2[c,e]	0.812	1.37	0.792	0.835
Race on V_2[e], % difference if black	88.4	33.0	36.7	151
Activity bias[g], % difference if OSA	-39.0	3.43	-41.6	-36.3
Activity bias[g], % difference if OSA local	-14.6	27.1	-22.4	-6.40
Assay on residual error[h], % difference if OSA	40.3	9.60	32.7	47.9
Inter-subject variability parameters[i]				
F[g], %CV	13.0	5.35	11.7	14.4
CL, %CV	30.5	7.75	26.3	35.6
Endogenous FVIII activity, %CV	7.19	15.8	4.95	9.34
Residual activity 1, %CV	119	5.98	106	132
Residual activity 2, %CV	152	8.43	128	178
Inter-occasion variability parameters[i]				
CL, %CV	34.7	5.25	31.8	38.6
V_2, %CV	41.0	10.1	33.1	49.1
Residual variability parameters[i]				
Proportional residual error, %CV	19.2	1.79	18.6	19.9
Proportional replicate error, %CV	10.4	3.91	9.63	11.3

AGE, age (years old); CI, confidence interval; CL, clearance; CV, coefficient of variation; F, bioavailability; FVIII, factor VIII; OSA, one-stage clotting assay; Q, inter-compartmental clearance; RSE, relative standard error; SIR, sampling importance resampling; V_1, volume of central compartment; V_2, volume of peripheral compartment; WT, weight (kg).

[a]RSE and CI for omega and sigma reported in the standard deviation scale. [b]Typical CL for a 20-year subject weighing 70 kg, being inhibitor negative and not belonging to study B1831090: $CL = 2.76 \times (WT / 70)^{3/4} \times$ age effect $\times (1 + 1.66 \times INH) \times (1 - 0.347 \times STUD)$, where INH=1 for inhibitors positive (otherwise = 0), STUD=1 for study B1831090 (otherwise = 0) and if AGE ≤1 year-old age effect $= (1 + 0.149 \times (AGE - 1) - (20 - 1) \times -0.00678) = 1.13 + 0.149 \times (AGE - 1)$, else age effect $= (1 - 0.00678 \times (AGE - 20))$. [c]Typical V_1 for a subject weighing 70 kg: $V_1 = 2.45 \times (WT / 70)^{0.81}$. [d]Typical Q for a subject weighing 70 kg: $Q = 25.1 \times (WT / 70)^{3/4}$. [e]Typical V_2 for a subject of non-black race weighing 70 kg: $V_2 = 0.923 \times (WT / 70)^{0.81} \times (1 + 0.884 \times RACE)$, where RACE=1 for black race (otherwise = 0). [f]Note, residual activity refers to FVIII activity measured before the first dose, i.e. residual activity from a previous dose not recorded. [g]F, the bioavailability term in NONMEM was used to adjust factor VIII activity as follows: Typical $F = 1 \times 1.38^{PROD} \times (1 - 0.390 \times METH1) \times (1 - 0.146 \times METH2)$, where PROD=1 for Xyntha (otherwise = 0), METH1 = 1 for OSA (otherwise = 0), METH2 = 1 for OSA local (otherwise = 0). [h]Change in CV% of overall proportional residual error. [i]η-shrinkage for inter-individual variability ranged between 24.6 and 86.3 % and η-shrinkage for inter-occasion variability was >53.9%; ε-shrinkage was 8.05% for sub-model 1 and 14.9% for sub-model 2.

Table 3. Overview of clinical trials included in the population PK analysis

Trial ID	Clinical Phase	Type of trial	Factor VIII product(s)	No. of patients[a]	Severity of hemophilia A[b]	Sampling design, samples/occasion[c]	Analytical assay	ClinicalTrials.gov identifier
B1831003	IV	safety surveillance	Xyntha	12	severe (<1 IU/dL)	sparse, 2	OSA	NCT00765726
B1831004 (28)	IV	safety surveillance	Refacto AF	206	severe (<1 IU/dL)	sparse, 2	CSA	NCT00884390
B1831015	III	safety and efficacy	Xyntha	53	severe (<1 IU/dL; N=12), moderately severe and moderate (1-5 IU/dL; N=40), mild[d] (5-40 IU/dL; N=1)	sparse, 2	OSA	NCT00868530
B1831053 (29)	III	safety and efficacy	Refacto	103	severe and moderately severe (<2 IU/dL)	rich, 11; sparse, 2	CSA	NA
B1831054 (30)	III	safety and efficacy	Refacto	91 (PUPs)	Severe and moderately severe (<2 IU/dL)	sparse, 4	CSA	NA
B1831061 (31)	IV	bioequivalence	Refacto	18	severe (<1 IU/dL)	rich, 11	CSA	NA
B1831066 (32)	III	pharmacokinetic	Refacto, Refacto AF	30[e]	severe (<1 IU/dL)	rich, 11-12[f]	CSA	NA
B1831067	III	safety and efficacy	Refacto AF	109[e]	Severe and moderately severe (<2 IU/dL)	rich, 11; sparse, 2	CSA	NCT00037544
B1831068	III	safety and efficacy	Refacto AF	96[e]	Severe and moderately severe (<2 IU/dL)	sparse, 2-3[f]	CSA	NA
B1831070 (33)	III	bioequivalence, safety and efficacy	Xyntha	92	severe (<1 IU/dL)	rich, 11; sparse, 1	OSA	NA
B1831071 (6)	III	safety and efficacy	Xyntha	30	Severe and moderately severe (<2 IU/dL)	rich, 11; sparse, 2-4[f]	OSA	NA
B1831077 (34)	I	pharmacokinetic	Refacto AF	16	Severe and moderately severe (<2 IU/dL)	rich, 11	CSA	NCT01579903
B1831090 (35)	I	bioequivalence	Refacto	18	Severe and moderately severe (<2 IU/dL)	rich, 12	CSA	NA
Total				874 (754 unique)				

CSA, chromogenic substrate assay; NA, not available; OSA, one-stage assay; PUPs, previously untreated patients.
[a]only patients contributing with one or more factor VIII activity observation were included. [b]severity of hemophilia depends on the endogenous factor VIII activity and the criterion of acceptance in each study is mentioned. [c]samples per occasion per patient including pre- and post-dose records; occasion defined as a visit that includes a sampling period. [d]the only patient with mild hemophilia enrolled in the trial was included in the population PK analysis. [e]some patients participated in more than one trial. [f]number of samples varied depending on the visit.