Population pharmacokinetics of recombinant coagulation factor VIII-SingleChain in patients with severe hemophilia A

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To cite this article: Zhang Y, Roberts J, Tortorici M, Veldman A, St Ledger K, Feussner A, Sidhu J. Population pharmacokinetics of recombinant coagulation factor VIII-SingleChain in patients with severe hemophilia A. J Thromb Haemost 2017; 15: 1106–14.

See also Berntorp E. If you know you will also see: population pharmacokinetics is the way to personalize and optimize prophylaxis in hemophilia. This issue, pp 1103–5.

Essentials

- rVIII-SingleChain is a unique recombinant factor VIII (FVIII) molecule.
- A population pharmacokinetic model was based on FVIII activity of severe hemophilia A patients.
- The model was used to simulate factor VIII activity–time profiles for various dosing scenarios.
- The model supports prolonged dosing of rVIII-SingleChain with intervals of up to twice per week.

Summary. Background: Single-chain recombinant coagulation factor VIII (rVIII-SingleChain) is a unique recombinant coagulation factor VIII molecule. Objectives: To: (i) characterize the population pharmacokinetics (PK) of rVIII-SingleChain in patients with severe hemophilia A; (ii) identify correlates of variability in rVIII-SingleChain PK; and (iii) simulate various dosing scenarios of rVIII-SingleChain. Patients/Methods: A population PK model was developed, based on FVIII activity levels of 130 patients with severe hemophilia A (n = 91 for ≥ 12–65 years; n = 39 for < 12 years) who had participated in a single-dose PK investigation with rVIII-SingleChain 50 IU kg⁻¹. PK sampling was performed for up to 96 h. Results: A two-compartment population PK model with first-order elimination adequately described FVIII activity. Body weight and predose level of von Willebrand factor were significant covariates on clearance, and body weight was a significant covariate on the central distribution volume. Simulations using the model with various dosing scenarios estimated that > 85% and > 93% of patients were predicted to maintain FVIII activity level above 1 IU dL⁻¹ at all times with three-times-weekly dosing (given on days 0, 2, and 4.5) at the lowest (20 IU kg⁻¹) and highest (50 IU kg⁻¹) doses, respectively. For twice weekly dosing (days 0 and 3.5) of 50 IU kg⁻¹ rVIII-SingleChain, 62–80% of patients across all ages were predicted to maintain a FVIII activity level above 1 IU dL⁻¹ at day 7. Conclusions: The population PK model adequately characterized rVIII-SingleChain PK, and the model can be utilized to simulate FVIII activity–time profiles for various dosing scenarios.

Keywords: factor VIII; hemophilia A; pharmacokinetics; recombinant proteins.

Introduction

Hemophilia A is a rare and serious X-linked recessive bleeding disorder that affects predominantly males and is characterized by a deficiency of coagulation factor VIII [1]. The recommended therapy for patients with hemophilia A is factor replacement therapy with a plasma-derived or recombinant coagulation FVIII (rFVIII) product, either in response to a bleeding episode (on-demand regimen) or to prevent bleeding (prophylaxis regimen). It has been shown that treatment of patients receiving a prophylaxis regimen is effective in reducing the number of bleeding episodes and preserving joint function [2]. Clinical guidelines for hemophilia A FVIII replacement therapy target FVIII activity levels of > 1 IU dL⁻¹ (> 1% of normal) [3], with the goal of changing the severity of hemophilia from severe
(endogenous FVIII activity of \(< 1\) IU dL\(^{-1}\)) to moderate (FVIII activity of \(> 1\) IU dL\(^{-1}\) to \(< 5\) IU dL\(^{-1}\)).

rVIII-SingleChain is a rFVIII molecule in which the heavy and light chains are covalently linked by a truncated B domain [4]. The novel design removes the furin cleavage site between the B and a3 domains, resulting in a single-chain molecule with a new N-glycosylation site. In vitro studies have shown that rVIII-SingleChain has a higher affinity for von Willebrand factor (VWF) than does rFVIII [5], and binds more quickly and more efficiently to VWF than does wild-type FVIII [6], leading to improved pharmacokinetic (PK) properties compared with full-length rFVIII [5,7]. Furthermore, in vitro data show that rVIII-SingleChain has a greater affinity for VWF than either NovoEight\(^8\) or the human cell line-derived recombinant human FVIII [8]. These favorable characteristics of rVIII-SingleChain could potentially overcome some of the major challenges in hemophilia care [7,9].

Considering the high affinity of rVIII-SingleChain for VWF, the current population PK analysis aimed to evaluate the impact of endogenous VWF binding to FVIII on PK parameters. Population PK modeling approaches constitute a standard analysis method for observed clinical PK data, accounting for relevant sources of variability, such as individual covariates of body weight (WT) or age [10,11]. Björkman et al. and Bolon previously described the relationships of FVIII pharmacokinetics (PK) to age and WT using a population PK model [12–14].

The current analysis utilized individual PK data from two clinical studies (Table 1) to develop a model to describe the population PK of rVIII-SingleChain in patients with severe hemophilia A, and to identify potential determinants (demographic and clinical covariates) of interindividual PK variability. Additionally, the population PK model was utilized to simulate FVIII activity with various routine prophylaxis dosing regimens of rVIII-SingleChain.

**Materials and methods**

**Study population**

A PK model was developed based on the levels of FVIII activity in patients aged 1–65 years who had FVIII activity \(< 1\) IU dL\(^{-1}\) and no detectable or history (personal or first-order relative) of FVIII inhibitors (neutralizing)

| Characteristic | CSL627_1001 | CSL627_3002 |
|---------------|-------------|-------------|
| Phase | Phase I/III | Phase III |
| Type of study | Open-label, multicenter, crossover, efficacy, PK | Open-label, safety, efficacy, PK |
| Age | \(\geq 12\) to \(\leq 65\) years | \(< 12\) years |
| Dose | Single injection of rVIII-SingleChain at 50 IU kg\(^{-1}\) | Single injection of rVIII-SingleChain at 50 IU kg\(^{-1}\) |
| WT (kg), median (range) | 72.2 (38.2–106) | 22.5 (10.0–87.0) |
| Age (years), median (range) | 29 (12–60) | 5 (1–11) |
| BMI (kg m\(^{-2}\)), median (range) | 23.7 (15.3–37.6) | 16.5 (12.8–29.8) |
| AST (IU L\(^{-1}\)), median (range) | 23.0 (12–199) | 31 (25–75) |
| ALT (IU L\(^{-1}\)), median (range) | 27 (8–142) | 19 (10–41) |
| VWF (% of normal), median (range) | 117 (57.6–296) | 96.0 (16.0–161) |
| CrCL (mg mL\(^{-1}\)), median (range) | 131.2 (61.8–221) | 167.3 (0.190–274) |
| HCT (%), median (range) | 44 (33.51.9) | 35 (31.5–40.6) |
| Hepatitis positivity* (N) | | |
| Yes | 32 | 0 |
| No | 59 | 39 |
| Non-neutralizing antidrug antibody against rVIII (N) | | |
| Present | 4 | 7 |
| Absent | 88 | 34 |
| Race (N) | | |
| White | 54 | 35 |
| Black or African American | 13 | 0 |
| Asian | 23 | 3 |
| Other | 1 | 1 |
| Region (N) | | |
| North America | 14 | 4 |
| Europe | 40 | 24 |
| Africa | 12 | 0 |
| Asia Pacific/Australia | 25 | 4 |
| Middle East | 0 | 7 |

ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CrCL, creatinine clearance; HCT, hematocrit; rVIII, recombinant factor VIII; VWF, von Willebrand factor; WT, body weight. *Includes hepatitis A, B, and C.
antibodies) at screening. All patients had received previous treatment for at least 150 exposure days (EDs) (adults and adolescents) or 50 EDs (children) with FVIII prior to enrollment. Patients had not received FVIII therapy for at least 4 days prior to being given rVIII-SingleChain at 50 IU kg$^{-1}$. Blood samples for PK assessments were collected before infusion (predose) and at 0.5, 1, 4, 8, 10, 24, 28, 48, 72 and, for some patients, up to 96 h after infusion for patients aged ≥ 12 years, and at predose and 1, 5, 10, 24 and 48 h after infusion for patients aged < 12 years.

**Sampling and bioanalytic methods**

A chromogenic assay was used to determine the FVIII activity in plasma samples. The assay was conducted in CSL Behring’s Central Laboratory (Marburg, Germany) with the Coamatic test kit (Chromogenix, Essen, Germany), by use of the Behring Coagulation System (Siemens Healthcare Diagnostics, Marburg, Germany). According to the current guidelines for analytic assay validation, the acceptance criteria of accuracy, precision, repeatability, linearity, range and robustness were predefined and assessed as passed in the validation program. The lower limit of quantification (LLOQ) was 1 IU dL$^{-1}$.

**Population PK model development**

A stepwise approach was used to construct the population PK model: the structural (compartmental PK) model was developed first, and this was followed by addition of the pharmacostatistical model, and finally the covariate model. The overall process of population PK model development was based on established regulatory guidance [15].

**Structural model** The structural model described the disposition (distribution and elimination) of rVIII-Single-Chain following intravenous administration, and also best characterized the PK data without consideration of interindividual variability (IIV). Model appropriateness was evaluated by the use of one-compartment, two-compartment and three-compartment PK models, and the best model was selected on the basis of the goodness of fit (GoF) and objective function value (OFV). Modeling was performed with the non-linear mixed-effect modeling software package NONMEM 7.2 (ICON Development Solutions, Ellicott City, MD, USA) [16]. R programming version 3.1.2 (R project open source; The R Foundation, Vienna, Austria; http://www.r-project.org) was used to generate the diagnostic plots.

For the FVIII activity PK models, endogenous FVIII activity levels were assumed not to be > 1 IU dL$^{-1}$, in order to maintain consistency with the prespecified study inclusion criteria. Endogenous FVIII activity and residual contributions from previous FVIII products to overall FVIII activity were estimated separately. Therefore, when patients had an observed FVIII activity of > 1 IU dL$^{-1}$ prior to receiving the first dose of rVIII-SingleChain, it was inferred that the observed plasma FVIII activity level represented a combination of: (i) endogenous FVIII activity; (ii) exponentially declining residual activity associated with previously administered FVIII products; and (iii) the net increase in FVIII activity levels resulting from administration of rVIII-SingleChain. However, if a patient’s predose FVIII activity level was < 1 IU dL$^{-1}$, it was considered to be related to endogenous activity only.

Missing drug concentrations were not imputed or accounted for in the model. Of 1714 samples assayed, 233 were below the assay LLOQ (BLQ), of which 130 samples were predose (as expected for patients with severe hemophilia A), and 103 were postdose and towards the end of the PK sampling period. No BLQ samples were reported between measurable concentrations. Therefore, all BLQ samples (6% of all samples) were omitted from the analysis [17].

**Pharmacostatistical model** The pharmacostatistical model comprised two components: the IIV model and the residual error model. The IIV model describes the unexplained random variability in individual values of the structural model parameters. IIV of the PK parameters was assumed to be log-normally distributed. IIV was estimated for clearance (CL), central distribution volume ($V_1$), and estimated endogenous FVIII activity. For the current PK analysis, the residual error model accounted for both additive and proportional error.

**Covariate model** Covariate assessment was performed to identify significant determinants of interindividual PK variability. Following development of the base model and estimation of individual model parameters, the relationship between covariates and IIV for CL and $V_1$ were investigated graphically prior to model significance testing. Covariates were selected on the basis of scientific interest, clinical interest, and/or prior knowledge. Two types of covariate (continuous and categorical) (Table 2) were evaluated for their effects on CL and $V_1$. A covariate was retained in the model if it significantly reduced the OFV (i.e. difference between the two OFVs of < 10.83 points; $P < 0.001$, 1 degree of freedom).

**Assessment of model adequacy** During model development, diagnostic plots, including predicted or individual predicted data versus observed data, and conditional weighted residuals versus time, were assessed. A visual predictive check (VPC) was performed to evaluate the performance of the final population PK model [18]. The VPC graphically assesses whether simulations from a model can reproduce the observed data. The median and the 5th and 95th percentiles of the concentration–time curves following 1000 simulations were superimposed with the observed data. A non-parametric bootstrap procedure was conducted to further investigate bias and precision of the parameter estimates.
Table 2 Covariates of interest

| Continuous covariates | Categorical covariates |
|-----------------------|------------------------|
| Body weight           | Hepatitis positivity   |
| Age                   | Presence/absence of antidrug |
| Body mass index       | antibody               |
| Aspartate aminotransferase | Geographical region |
| Alanine aminotransferase | Race                      |
| Creatinine clearance  | Japanese versus non-Japanese |
| Baseline von Willebrand factor | Age ≥ 12 years versus < 12 years |
| Hematocrit            | Initial PK assessment versus repeat PK assessment |

PK, pharmacokinetic.

Simulations

The final FVIII activity population PK model was used to simulate FVIII activity–time profiles (i) in patients given single doses of 20, 30, 40 and 50 IU kg⁻¹ rVIII-SingleChain, and (ii) at steady state following multiple dosing scenarios, i.e. 20, 30, 40 and 50 IU kg⁻¹ intravenous administration every 2 days, every 3 days, twice weekly (dosing every 3.5 days or on days 0 and 3), and three times weekly (dosing on days 0, 2, and 4.5, or on days 0, 2, and 4). For these simulations, the individual WT and VWF levels of the patients included in the population PK modeling were used in the simulation dataset, with a 10-min infusion time being assumed. For each simulation scenario, 1000 replicates of the simulation datasets were performed. The median and 90% prediction intervals of the simulated FVIII activity–time profiles were calculated and constructed graphically for different dosing regimens.

For single-dose simulations, the time needed to maintain FVIII activity above 1 IU dL⁻¹ was predicted. For multiple-dose simulations, the FVIII steady-state median (90% prediction intervals) activity and the percentage of patients with activity above 1 IU dL⁻¹ at the end of the dosing interval were derived for each dosing regimen. Although no BLQ data were utilized in the model development, the model-based simulations may, on occasion, have predicted activity levels below the assay LLOQ, related to the variability of baseline estimation.

Results

PK model development

The data analyzed were best described by a two-compartment PK model that included structural parameters for CL, \( V_1 \), intercompartmental clearance, peripheral volume of distribution, endogenous FVIII activity, and residual contributions from previously administered FVIII products. IIV was estimated for CL, \( V_1 \) and endogenous FVIII activity only, although no covariate analysis was conducted on endogenous FVIII activity. A thorough covariate analysis for CL and \( V_1 \) indicated that WT and VWF were significant descriptors of CL, and that WT was a significant descriptor of \( V_1 \). The final model incorporated these significant covariates and accounted for the correlation between CL and \( V_1 \). An additional analysis was also performed to examine the correlation between WT-adjusted CL and that of WT-adjusted \( V_1 \) and volume of distribution at steady state. The correlations are shown in Fig. S1.

All parameters of the final PK model (Table 3) were estimated with reasonable precision (relative standard error of the estimate of < 26%). The shrinkage in IIV estimates (2.13%, 5.18% and 60.6% for CL, \( V_1 \) and endogenous FVIII activity, respectively) indicated a reasonable distribution for the estimation of subject-specific random effects.

The final FVIII population PK model equations for CL and \( V_1 \) were as follows:

\[
CL = 2.12 \times \left(\frac{WT}{68}\right)^{0.756} \times \left(\frac{VWF}{113}\right)^{-0.633} \quad (1)
\]

\[
V_1 = 33.6 \times \left(\frac{WT}{68}\right)^{0.903} \quad (2)
\]

Model validation

On the basis of the VPC, the final FVIII activity population PK model provided a good description of the data. Observed median FVIII activity concentrations, and the observed 5th and 95th percentiles, were consistently

| Parameter                         | NONMEM estimates |
|-----------------------------------|------------------|
|                                  | Point estimate   | % RSE  | 95% CI               |
| CL (dL h⁻¹)                       | 2.12             | 2.70   | 2.02 to 2.23         |
| WT effect on CL                   | 0.756            | 4.72   | 0.686 to 0.825       |
| VWF effect on CL                  | -0.633           | 8.36   | -0.740 to -0.526     |
| \( V_1 \) (dL)                     | 33.6             | 2.05   | 32.2 to 35.0         |
| WT effect on \( V_1 \)            | 0.903            | 3.46   | 0.840 to 0.986       |
| \( Q \) (dL h⁻¹)                   | 1.34             | 25.6   | 0.338 to 2.33        |
| \( V_2 \) (dL)                     | 2.65             | 9.15   | 2.01 to 3.29         |
| BASE (IU dL⁻¹)                    | 0.765            | 22.0   | 0.395 to 1.13        |
| Interindividual variability*      |                  |        |                      |
| \( \sigma^2_{CL} \)               | 0.0583           | 6.90   | 0.0423 to 0.0743     |
| \( \sigma^2_{V1} \)               | 0.0388           | 9.09   | 0.0239 to 0.0537     |
| \( \sigma^2_{BASE} \)             | 0.334            | 20.8   | -0.211 to 0.879      |
| Residual variability†             |                  |        |                      |
| \( \sigma^2_{prop} \)             | 0.109            | 14.3   | 0.0810 to 0.138      |
| \( \sigma^2_{add} \)              | 1.15             | 16.3   | 0.753 to 1.54        |

BASE, endogenous FVIII activity; CI, confidence interval on the parameter from bootstrap (\( n = 1000 \)); CL, clearance; NONMEM, non-linear mixed-effect modeling software; \( Q \), intercompartmental clearance; RSE, relative standard error of the estimate; \( V_2 \), volume of central compartment; \( V_2 \), volume of peripheral compartment; VWF, von Willebrand factor; WT, body weight; \( \sigma^2 \), variance of random effect; \( \sigma^2_{prop} \), proportional component of the residual error model; \( \sigma^2_{add} \), additive component of the residual error model.

*Values shown represent the variance of random effect. †Values shown represent either the proportional or additive components of the residual error model. The reference population weight for the pharmacokinetic parameters for \( V_1 \) and \( V_2 \) is 70 kg.
within the 90% prediction intervals for each parameter derived from the PK model (Fig. 1). Moreover, after stratification by age (< 12 years and ≥ 12 years), the VPC confirmed that the model adequately described the PK for the two age groups (Fig. S2). In addition, the GoF plots described the observed data with no systematic bias in model predictions (Fig. S3).

**PK simulations**

The final PK model was used to determine the (population) probability and (individual) durations above a target 1 IU dL\(^{-1}\) level of FVIII activity, evaluated separately for the different age groups: < 6 years, ≥ 6 to < 12 years, and ≥ 12 years.

Simulations were performed according to two key assumptions: (i) PK linearity at doses between 20 and 50 IU kg\(^{-1}\); and (ii) the endogenous FVIII activity levels not being > 1 IU dL\(^{-1}\) FVIII activity in order to be consistent with both the plasma level in severe hemophilia A (FVIII activity of < 1 IU dL\(^{-1}\)) and the inclusion criteria for both studies.

**Single-dose simulations**

The median (90% prediction interval) and 25th percentile duration for which FVIII activity (which included endogenous FVIII activity and rVIII-SingleChain activity) was maintained above 1 IU dL\(^{-1}\) was 20–50 IU kg\(^{-1}\) dose scenarios are shown in Table 4 for the overall study population (pediatrics and adults).

**Multiple-dose/steady-state simulations**

Table 5 shows the median (90% prediction intervals) and 25th percentile of the FVIII activity level (which included endogenous FVIII activity and rVIII-SingleChain activity) at the end of the dosing interval for steady-state simulations, and the percentage of patients predicted to maintain an FVIII activity level above 1 IU dL\(^{-1}\) in all age groups.

The median simulated FVIII activity was maintained above 1 IU dL\(^{-1}\) for 4.3, 4.6, 4.9 and 5.1 days for single doses of 20, 30, 40 and 50 IU kg\(^{-1}\), respectively. The trough FVIII activity and the percentage of patients who maintained FVIII activity levels above 1 IU dL\(^{-1}\) at the end of the dosing interval in steady-state simulations was predicted for multiple-dosing regimens of rVIII-SingleChain. For three-times-weekly dosing (days 0, 2, and 4.5) with 50 IU kg\(^{-1}\) rVIII-SingleChain, 86–96% of patients across all age groups were predicted to maintain an FVIII activity level above 1 IU dL\(^{-1}\) at day 7. For twice weekly dosing (days 0 and 3.5) with 50 IU kg\(^{-1}\) rVIII-SingleChain, 62–80% of patients across all age groups were predicted to maintain an FVIII activity level above 1 IU dL\(^{-1}\) at day 7.

The predicted steady-state total FVIII activity profiles for rVIII-SingleChain in different dosing scenarios for all age groups are shown in Fig. 2 (20 IU kg\(^{-1}\) and 50 IU kg\(^{-1}\) twice weekly) and Fig. 3 (20 IU kg\(^{-1}\) and 50 IU kg\(^{-1}\) three times weekly). Simulated PK curves for nine individual patients compared with observed FVIII activity levels over time are shown in Fig. S4.

**Discussion**

A population PK model for rVIII-SingleChain was developed on the basis of data from pediatric and adult patients with severe hemophilia A participating in clinical studies.

**Table 4** Summary of the simulated durations of factor VIII activity that were maintained above 1 IU dL\(^{-1}\) following a single dose of 20, 30, 40 or 50 IU dL\(^{-1}\) of rVIII-SingleChain

| Simulated single intravenous dose (IU kg\(^{-1}\)) | Median (days) | 25th percentile (days) | 90% PI (days) |
|-----------------------------------------------|---------------|------------------------|---------------|
| 20                                           | 4.3           | 2.9                    | 1.9–NA        |
| 30                                           | 4.6           | 3.2                    | 2.1–NA        |
| 40                                           | 4.9           | 3.4                    | 2.3–NA        |
| 50                                           | 5.1           | 3.5                    | 2.4–NA        |

NA, not able to be calculated; PI, prediction interval. One thousand replicates of 130 patients were used for each simulation.
studies. Population PK models have been previously demonstrated to constitute a useful methodology for the PK characterization of a rFVIII product [12]. The current population PK model was best described by a two-compartment model with linear elimination (on a logarithmic scale) that included a population estimate of baseline FVIII activity. The model estimated a mean baseline FVIII activity of < 1 IU dL\(^{-1}\), which is consistent with the inclusion criteria for patients with severe hemophilia A (< 1 IU dL\(^{-1}\)) in the clinical studies of rVIII-SingleChain. The model identified key sources of variability in the clearance and distribution volume of rVIII-SingleChain, and was used to simulate trough levels of FVIII activity for different clinically relevant dose regimens of rVIII-SingleChain in patients with severe hemophilia A.

WT, as a covariate, was a key descriptor of the variability in rVIII-SingleChain PK, and was correlated

| Regimen dose and administration | PK sampling (day) | Age group (years) | Median trough (90% PI) (IU dL\(^{-1}\)) | Percentage of patients maintaining > 1 IU dL\(^{-1}\) rVIII-SingleChain FVIII activity level |
|---------------------------------|------------------|-----------------|----------------------------------|-----------------------------------|
| 20 IU kg\(^{-1}\) twice weekly, on days 0 and 3.5 | 3.5, 7 | < 6 | 1.0 (0.39–2.4) | 49.3 |
| | | 6 to < 12 | 1.0 (0.40–2.6) | 52.1 |
| | | ≥ 12 | 1.3 (0.48–3.9) | 67.8 |
| 50 IU kg\(^{-1}\) twice weekly, on days 0 and 3.5 | 3.5, 7 | < 6 | 1.2 (0.45–3.4) | 62.3 |
| | | 6 to < 12 | 1.3 (0.45–4.1) | 65.2 |
| | | ≥ 12 | 1.9 (0.58–8.0) | 80.4 |
| 20 IU kg\(^{-1}\) three times weekly, on day 0 | 2 | < 6 | 2.0 (0.71–4.8) | 86.9 |
| | | 6 to < 12 | 2.3 (0.78–6.0) | 89.7 |
| | | ≥ 12 | 3.5 (1.1–9.5) | 96.5 |
| 20 IU kg\(^{-1}\) three times weekly, on day 2 | 4.5 | < 6 | 1.4 (0.54–3.7) | 72.8 |
| | | 6 to < 12 | 1.6 (0.57–4.6) | 76.9 |
| | | ≥ 12 | 2.4 (0.76–7.8) | 89.3 |
| 20 IU kg\(^{-1}\) three times weekly, on day 4.5 | 7 | < 6 | 1.4 (0.54–3.6) | 72.6 |
| | | 6 to < 12 | 1.6 (0.57–4.4) | 76.7 |
| | | ≥ 12 | 2.4 (0.76–7.5) | 89.1 |
| 50 IU kg\(^{-1}\) three times weekly, on day 0 | 2 | < 6 | 3.6 (0.92–10.2) | 94.1 |
| | | 6 to < 12 | 4.2 (1.1–13.3) | 96.4 |
| | | ≥ 12 | 7.5 (1.8–22.1) | 99.1 |
| 50 IU kg\(^{-1}\) three times weekly, on day 2 | 4.5 | < 6 | 2.2 (0.67–7.3) | 86.3 |
| | | 6 to < 12 | 2.5 (0.73–9.6) | 88.6 |
| | | ≥ 12 | 4.5 (1.1–17.9) | 95.8 |
| 50 IU kg\(^{-1}\) three times weekly, on day 4.5 | 7 | < 6 | 2.2 (0.67–7.1) | 86.2 |
| | | 6 to < 12 | 2.5 (0.73–9.3) | 88.6 |
| | | ≥ 12 | 4.4 (1.1–17.3) | 95.8 |

PI, prediction interval; PK, pharmacokinetic. The lower limit of quantification (LLOQ) for the chromogenic assay is 1 IU dL\(^{-1}\). Simulated activity may be below the LLOQ.

Fig. 2. Predicted steady-state factor VIII activity profiles for rVIII-SingleChain at two different dosing schedules: 20 IU kg\(^{-1}\) (red) and 50 IU kg\(^{-1}\) (blue) twice weekly for all age groups. The y-axis represents FVIII activity on a logarithmic scale. Dashed lines: median predicted values. Shaded regions: 90% prediction intervals. Red horizontal dashed lines: 1 IU dL\(^{-1}\) FVIII activity.

Table 5 Summary of the simulated steady-state trough factor VIII activity following multiple doses of 20 or 50 IU kg\(^{-1}\) of rVIII-SingleChain
Predicted steady-state factor VIII activity profiles for rVIII-SingleChain at two different dosing schedules: 20 IU kg\(^{-1}\) (red) and 50 IU kg\(^{-1}\) (blue) three times weekly for all age groups. The y-axis represents FVIII activity on a logarithmic scale. Dashed lines: median predicted values. Shaded regions: 90% prediction intervals. Red horizontal dashed lines: 1 IU dL\(^{-1}\) FVIII activity.

Fig. 3. Predicted steady-state factor VIII activity profiles for rVIII-SingleChain at two different dosing schedules: 20 IU kg\(^{-1}\) (red) and 50 IU kg\(^{-1}\) (blue) three times weekly for all age groups. The y-axis represents FVIII activity on a logarithmic scale. Dashed lines: median predicted values. Shaded regions: 90% prediction intervals. Red horizontal dashed lines: 1 IU dL\(^{-1}\) FVIII activity.

significantly with both CL and V\(_f\). Inclusion of pediatric patients in the model provided a wide WT range (10–106 kg) in the dataset, thereby enabling the estimation of allometric exponents. The effect of WT on rVIII-SingleChain CL was estimated with an allometric exponent of 0.756, which is similar to the common clinical pediatric weight-scaling exponent value of 0.75 for CL [19]. The exponential relationship observed between rVIII-SingleChain CL and WT is consistent with the observed FVIII PK profiles, where CL was higher (on a per kg WT basis) in the lighter patients. Age, in addition to WT, has previously been reported to be an important covariate of PK in patients with severe hemophilia A [12]. However, incorporating an age effect in addition to WT on CL in the present analysis did not provide further improvement of the model or explain the additional variability in the parameter estimates. A likely explanation for the apparent lack of an effect of age is the strong correlation between age and WT in the pediatric population, leading to colinearity. WT was also identified as a significant covariate on V\(_f\), with an estimated allometric exponent of 0.903, which is close to the theoretical value of 1.0 in scaling PK distribution volumes [20].

Baseline VWF was also a major covariate on CL of rVIII-SingleChain, with increasing VWF levels being associated with lower CL. This finding is consistent with a previous PK study examining the effects of VWF levels on FVIII PK in patients with severe hemophilia A. In that study, increasing levels of endogenous VWF were correlated with lower CL and an extended half-life of FVIII [21]. However, prior to the current analysis, the impact of VWF on FVIII PK had not previously been evaluated with population PK analysis. To illustrate the magnitude of this impact, our model predicts that a 68-kg subject with a baseline VWF value of either 113% or 200% of normal would have a rVIII-SingleChain CL of 2.12 dL h\(^{-1}\) or 1.48 dL h\(^{-1}\), respectively. Thus, in the case of a high (200% of normal) baseline VWF level, CL would be reduced by around 30% compared with the population estimate of a typical baseline value (113%) of VWF.

Prophylactic dosing strategies for patients with hemophilia A are based primarily on the patient’s clinical phenotype and maintaining FVIII activity above a trough level of >1 IU dL\(^{-1}\) [22]. This strategy is derived from evidence showing that increased time spent with a FVIII activity level of <1 IU dL\(^{-1}\) is linked to increased breakthrough bleeding during prophylactic treatment [23]. With the developed rVIII-SingleChain PK model, simulations showed that FVIII activity would be maintained above 1 IU dL\(^{-1}\) for 5.1 days following a single dose of 50 IU kg\(^{-1}\) rVIII-SingleChain. Simulations in the current rVIII-SingleChain analysis also demonstrated that the majority of the patients would be expected to maintain FVIII levels above 1 IU dL\(^{-1}\) with twice weekly and three times weekly dosing across a dose range of 20–50 IU kg\(^{-1}\). The twice weekly dosing regimen offers the significant clinical advantage in the pediatric population of avoiding early-morning intravenous infusions during the week. For example, dosing every 3.5 days would allow infusions on Wednesday evening and Sunday morning, with most of the patients maintaining FVIII trough levels above 1 IU dL\(^{-1}\).

The application of this model is not limited to PK predictions; it also has the potential to predict individualized dosing and estimate individual half-life. Prior publications have utilized a limited number of PK samples with patient characteristics (e.g., WT) and have employed Bayesian estimation approaches [24]. A potential limitation of the current analysis is that the dose regimens used in the simulations were not identical to those used in the clinical studies for observed PK characterization. The FVIII simulations, which were performed over the dose range of 20–50 IU kg\(^{-1}\), were based primarily on observed PK linearity over a dose range of 45–60 IU kg\(^{-1}\). The assumption of PK linearity is further supported by data from
other rFVIII replacement therapies (rFVIII:Fc and Advate\textsuperscript{b}) that have shown approximately linear PK between the doses of 25 and 65 IU kg\textsuperscript{-1} \cite{25}

In conclusion, the population PK model developed here characterized the PK of rVIII-SingleChain as a function of patient endogenous FVIII activity, WT, and VWF levels. The PK parameters estimated by the model correlated well with the observed clinical PK data, indicating that this model is suitable for predicting FVIII activity levels in patients treated with rVIII-SingleChain at a population level and a subpopulation (e.g. pediatric) level. The variability in FVIII activity was not significantly associated with any demographic or clinical covariate, apart from WT and VWF level. The developed model was utilized for extensive dosing regimen evaluation, confirming that the administration of rVIII-SingleChain 20–50 IU kg\textsuperscript{-1} twice or three times weekly is an acceptable treatment option for patients with severe hemophilia A.

Addendum

A. Veldman and K. St Ledger contributed to the concept, design and execution of the clinical study. Y. Zhang, J. Roberts, M. Tortorici, A. Feussner, and J. Sidhu analyzed and interpreted the data. Y. Zhang and J. Roberts wrote the paper. M. Tortorici, J. Sidhu, A. Veldman, and K. St Ledger revised the intellectual content. All authors approved the final version.

Acknowledgements

The authors thank all of the patients and their families who contributed to this study, and all of the participating investigators, research nurses, and data coordinators.

Disclosure of Conflict of Interests

This article was financially supported by CSL Behring, USA. A. Feussner, J. Roberts, J. Sidhu, K. St Ledger, M. Tortorici, A. Veldman, and Y. Zhang are employees of CSL Behring.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Individual data points for rVIII-SingleChain clearance (CL) versus volume of central compartment ($V_1$) (left panel) and volume of distribution at steady state ($V_{ss}$) (right panel); adjusted for weight (WT).

**Fig. S2.** Visual predictive check for the final factor VIII (FVIII) activity population pharmacokinetic model for patients aged < 12 years (left panel) and ≥ 12 years (right panel).

**Fig. S3.** Goodness of fit plots of the final population pharmacokinetic model.

**Fig. S4.** Observed (red open circles) and model-predicted (green solid line) FVIII activity–time profiles of patients receiving 50 IU kg\textsuperscript{-1} rVIII-SingleChain.

References

1. Bolton-Maggs PHB, Pasi KJ. Haemophilias A and B. *Lancet* 2003; 361: 1801–9.
2. Manco-Johnson MJ, Abshire TC, Shapiro AD, Riske B, Hacker MR, Kilcoyne R, Ingram JD, Manco-Johnson ML, Funk S, Jacobson L, Valentino LA, Hoots WK, Buchanan GR, DiMichele D, Recht M, Brown D, Leissinger C, Bleak S, Cohen A, Mathew P, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med* 2007; 357: 535–44.
3. Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, Ludlam CA, Mahlangu JN, Mulder K, Poon MC, Street A. Guidelines for the management of hemophilia. *Haemophilia* 2012; 19: e1–47.
4. Schmidbauer S, Witzel R, Robbel L, Sebastian P, Grammel N, Metzner HJ, Schulte S. Physicochemical characterisation of rVIII-SingleChain, a novel recombinant single-chain factor VIII. *Thromb Res* 2015; 136: 388–95.
5. Zollner S, Raquet E, Claar P, Müller-Cohrs J, Metzner HJ, Weimer T, Pragst I, Dickneite G, Schulte S. Non-clinical pharmacokinetics and pharmacodynamics of rVIII-SingleChain, a novel recombinant single-chain factor VIII. *Thromb Res* 2014; 134: 125–31.
6. Zollner SB, Raquet E, Müller-Cohrs J, Metzner HJ, Weimer T, Pragst I, Dickneite G, Schulte S. Preclinical efficacy and safety of rVIII-SingleChain (CSL627), a novel recombinant single-chain factor VIII. *Thromb Res* 2013; 128: 280–7.
7. Klarmuth R, Simpson M, von Depta-Prondzinski M, Gill JC, Morfini M, Powell JS, Santagostino E, Davis J, Huth-Kühne A, Leissinger C, Neumeister P, Bensen-Kennedy D, Feussner A, Limaskun T, Zhou M, Veldman A, Ledger KS, Blackman N, Pabinger I. Comparative pharmacokinetics of rVIII-SingleChain and octocog alfa (Advate\textsuperscript{b}) in patients with severe haemophilia A. *Haemophilia* 2016; 22: 730–8.
8. Sandberg H, Kannicht C, Stenlund P, Dadaian M. Functional characteristics of the novel, human-derived recombinant FVIII protein product, human-crl rhFVIII. *Thromb Res* 2012; 130: 808–17.
9. Mahlangu J, Kuliczkowski K, Karim FA, Stussyhyn O, Kosinova MV, Lepatan LM, Skotnicki A, Boggio LN, Klarmoth R, Oldenbrug J, Hellmann A, Santagostino E, Baker RI, Fischer K, Gill JC, P’Ng S, Chowdry P, Escobar MA, Khayat CD, Rusen L, et al. Efficacy and safety of rVIII-SingleChain: results of a phase 1/3 multicenter clinical trial in severe hemophilia A. *Blood* 2016; 128: 630–7.
10. Sheiner LB. The population approach to pharmacokinetic data analysis: rationale and standard data analysis methods. *Drug Metab Rev* 1984; 15: 153–71.
11. Aarons L. Population pharmacokinetics: theory and practice. *Br J Clin Pharmacol* 1991; 32: 669–70.
12. Björkman S, Oh MS, Spotts G, Schrotz P, Fritsch S. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. *Blood* 2012; 119: 612–18.
13. Bolon M. A limited sampling strategy for estimating individual pharmacokinetic parameters of coagulation factor VIII in patients with hemophilia A. *Ther Drug Monit* 2007; 29: 20–6.
14 Björkman S. Comparative pharmacokinetics of factor VIII and recombinant factor IX: for which coagulation factors should half-life change with age? Haemophilia 2013; 19: 882–6.

15 Population Pharmacokinetic Working Group. Guidance for industry: population pharmacokinetics. February 1999. Silver Spring, MD: US Center for Drug Evaluation and Research. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072137.pdf. Accessed 28 August 2016.

16 Bauer RJ. NONMEM Users Guide: Introduction to NONMEM 7.2.0. Ellicott City, MD: ICON Development Solutions; 2011.

17 Keizer RJ, Jansen RS, Rosing H. Incorporation of concentration data below the limit of quantification in population pharmacokinetic analyses. Pharmacol Res Perspect 2015; 3: e00131.

18 Karlsson MO, Savic RM. Diagnosing model diagnostics. Clin Pharmacol Ther 2007; 82: 17–20.

19 Anderson BJ, Holford NHG. Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab Pharmacokinet 2009; 24: 25–36.

20 Mordenti J. Man versus beast: pharmacokinetic scaling in mammals. J Pharm Sci 1986; 75: 1028–40.

21 Lalezari S, Martinowitz U, Windyga J, Enriquez MM, Delesen H, Schwartz L, Scharrer I. Correlation between endogenous VWF:Ag and PK parameters and bleeding frequency in severe haemophilia A subjects during three-times-weekly prophylaxis with rFVIII-FS. Haemophilia 2013; 20: e15–22.

22 Jiménez-Yuste V, Auerswald G, Benson G, Lambert T, Morfini M, Remor E, Salek SZ. Achieving and maintaining an optimal trough level for prophylaxis in haemophilia: the past, the present and the future. Blood Transfus 2014; 12: 314–19.

23 Collins PW, Blanchette VS, Fischer K, Björkman S, Oh M, Fritsch S, Schroth P, Spotts G, Astemark J, Ewenstein B; on behalf of the RAHF-PFM Study Group. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. J Thromb Haemost 2009; 7: 413–20.

24 Björkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. Haemophilia 2010; 16: 597–605.

25 European Medicines Agency (EMA). Committee for Medicinal Products for Human Use (CHMP) Assessment Report for ELOCTA. Document Reference EMA/671791/2015. London: EMA, 2015.