

ORIGINAL ARTICLE

Safety, efficacy and pharmacokinetics of rVIII-SingleChain in children with severe hemophilia A: results of a multicenter clinical trial

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Essentials

- rVIII-SingleChain is a novel recombinant factor VIII with covalently bonded heavy and light chains.
- Efficacy, safety and pharmacokinetics were studied in pediatric patients with severe hemophilia A.
- Across all prophylaxis regimens, the median annualized spontaneous bleeding rate was 0.00.
- rVIII-SingleChain showed excellent hemostatic efficacy and a favorable safety profile.

Summary. *Background:* rVIII-SingleChain is a novel B-domain truncated recombinant factor VIII (rFVIII) comprised of covalently bonded FVIII heavy and light chains, demonstrating a high binding affinity to von Willebrand factor. *Objectives:* This phase III study investigated the safety, efficacy and pharmacokinetics of rVIII-SingleChain in previously treated pediatric patients < 12 years of age with severe hemophilia A. *Patients/Methods:* Patients could be assigned to prophylaxis or on-demand therapy by the investigator. For patients assigned to prophylaxis, the treatment regimen and dose were based on the bleeding phenotype. For patients receiving

on-demand therapy, dosing was guided by World Federation of Hemophilia recommendations. The primary endpoint was treatment success, defined as a rating of ‘excellent’ or ‘good’ on the investigator’s clinical assessment of hemostatic efficacy for all treated bleeding events. *Results:* The study enrolled 84 patients (0 to < 6 years, n = 35; ≥ 6 to < 12 years, n = 49); 81 were assigned to prophylaxis and three to an on-demand regimen. Patients accumulated a total of 5239 exposure days (EDs), with 65 participants reaching > 50 EDs. In the 347 bleeds treated and evaluated by the investigator, hemostatic efficacy was rated as excellent or good in 96.3%. The median annualized spontaneous bleeding rate was 0.00 (Q1, Q3: 0.00, 2.20), and the median annualized bleeding rate was 3.69 (Q1, Q3: 0.00, 7.20) across all prophylaxis regimens. No participant developed an inhibitor. *Conclusions:* rVIII-SingleChain is a novel rFVIII molecule showing excellent hemostatic efficacy and a favorable safety profile in a clinical study in children < 12 years of age with severe hemophilia A.

Keywords: clinical trial; factor VIII; hemophilia A; pediatric; pharmacokinetics; safety.

Introduction

rVIII-SingleChain is a novel recombinant factor VIII (rFVIII) in which the heavy and light chains are covalently fused to achieve a single polypeptide protein. Upon activation by thrombin, rVIII-SingleChain is indistinguishable from endogenous activated FVIII [1,2]. The single-chain design results in a stable and homogenous drug

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product with an increased binding to von Willebrand factor (VWF), an attribute shown to be a key determinant in FVIII half-life as well as a mechanism to mitigate the risk of inhibitor development [2,3].

The increased affinity of rVIII-SingleChain to VWF improved the pharmacokinetics (PK) of the molecule, as shown in previous investigations in adults and adolescents with severe hemophilia A, in which rVIII-SingleChain demonstrated superior PK properties when compared with those of full-length rFVIII [4]. A large study investigating rVIII-SingleChain in 175 adult and adolescent patients with 14 306 exposure days (EDs) demonstrated excellent efficacy of rVIII-SingleChain in the control of bleeding events and in routine and surgical prophylaxis. No patient developed an inhibitor while in the study [5].

Here, we report on the efficacy, safety and PK results of a prospective phase III study investigating rVIII-SingleChain in children < 12 years of age with severe hemophilia A (ClinicalTrials.gov Identifier: NCT02093897).

Patients and methods

Patients

This study recruited patients with severe hemophilia A (FVIII activity < 1%), with > 50 previous EDs to FVIII prior to enrollment and aged between 0 and < 12 years. Patients with a history (personal or first-grade relatives) of FVIII inhibitors or a detectable inhibitor titer at screening were excluded. Following the European Medicines Agency guidance on the development of novel FVIII products [6], the study aimed to recruit a minimum of 75 patients to ensure that at least 25 subjects in each age group (0 to < 6 years and ≥ 6 to < 12 years) received 50 EDs of rVIII-SingleChain. The study was conducted in accordance with the International Conference on Harmonization Guidelines for Good Clinical Practice and the ethical principles outlined in the Declaration of Helsinki 2008 [7]. Approval by the Institutional Review Boards and the relevant national authorities and individual written informed consent/assent were obtained prior to enrollment.

Dosing

Patients were assigned to either prophylaxis or on-demand therapy by the investigator; switching therapies was not permitted during the study. At the investigator's discretion, for prophylaxis treatment, subjects received rVIII-SingleChain at a dose of 15 to 50 IU kg⁻¹ every second day or two to three times per week or at a dose and frequency determined by the investigator based on historical FVIII dosing and available PK data. For the treatment of breakthrough bleeds in patients on routine prophylaxis and for the treatment of bleeds in patients assigned to an on-demand regimen, dosing of rVIII-

SingleChain was guided by World Federation of Hemophilia recommendations [8]. The dose and frequency could be adjusted during the study if necessary.

Efficacy assessments

All bleeding events in the study treated with rVIII-SingleChain were rated by the investigator on a 4-point rating scale for hemostatic efficacy during the patients' monthly visits at the study site (every 3 months after the first 6 months on study), based on his or her clinical judgment and informed by information provided by the patient and documentation available in the eDiary on the number of doses needed to control the bleed (Table 1). Ratings of excellent or good were considered as treatment success.

The bleeding rate was calculated for all bleeds (annualized bleeding rate [ABR]), for spontaneous bleeding events (annualized spontaneous bleeding rate [AsBR]) and for joint bleeds.

Safety assessments

Safety was assessed on the basis of the following variables: number, type and severity of adverse events (AEs), development of inhibitors against FVIII, development of non-inhibitory anti-drug-antibodies (ADA) and anti-Chinese hamster ovary (CHO) host cell protein antibodies, vital signs and physical examination, laboratory safety parameters (hematology and biochemistry), and local tolerability at the site of infusion assessed by the investigator and patient.

Inhibitor testing

Inhibitor tests were performed at screening, after 10–15 EDs and after 50–75 EDs (at the subsequent monthly

Table 1 Evaluation of efficacy in the treatment of bleeding events; 4-point rating scale

Rating	Criteria
Excellent	Definite pain relief and/or improvement in signs of bleeding (i.e. swelling, tenderness and/or increased range of motion in the case of musculoskeletal hemorrhage) within approximately 8 h after the first rVIII-SingleChain infusion.
Good	Definite pain relief and/or improvement in signs of bleeding at approximately 8 h after the first rVIII-SingleChain infusion; requires two infusions for complete resolution.
Moderate	Probable or slight beneficial effect within approximately 8 h after the first rVIII-SingleChain infusion; requires more than two infusions for complete resolution.
Poor/no response	No improvement at all or condition worsens (i.e. signs of bleeding) after the first rVIII-SingleChain infusion and additional hemostatic intervention is required with another FVIII product, cryoprecipitate, or plasma for complete resolution.

visit). In addition, any subject that had an end of study visit had a test for inhibitors at that visit, independent of EDs. Additional tests could be triggered by the investigator at any time. All samples were assessed with the Nijmegen assay in the central laboratory. A subject was considered to have developed a positive inhibitor if a sample assayed at the central laboratory using the Nijmegen assay yielded a result of ≥ 0.6 BU mL $^{-1}$ and that finding was confirmed in a second, independent sample.

PK assessment

A non-compartmental PK analysis of FVIII activity in plasma was performed with and without baseline correction for the individual participant plasma FVIII activity-vs.-time data using Model 202 for constant infusion in WinNonlin® 6.3.0 (Phoenix Build 6.3.0.395, Pharsight Corp., St Louis, MO, USA). Following a 4-day washout period, blood samples for PK assessments were collected immediately before injection and 1, 5, 10, 24 and 48 h after injection, following the International Society of Thrombosis and Haemostasis recommendations. FVIII activity was measured in the blood samples using both the chromogenic substrate (ChS) and one-stage clotting (OS) assays. Baseline correction of FVIII activity was performed by subtracting the participant's pre-dose activity level from the activity level obtained at each time-point after dosing. The FVIII activity level before rVIII-SingleChain dosing was used as the baseline for PK assessment of rVIII-SingleChain. Actual sampling times, doses (according to the corresponding potency of the specific lot numbers and the injection volumes) and duration of injection were used for calculation of PK parameters.

The potency of rVIII-SingleChain was assigned using the ChS assay calibrated against the World Health Organization FVIII standard. In line with other B-domain altered FVIII products, there is a discrepancy between results of the OS assay and the ChS assay for rVIII-SingleChain. Pre-clinical studies and *in vitro* characterization confirmed the ChS assay is most representative of the expected clinical effectiveness, whereas the one-stage assay underestimates the FVIII plasma activity in patients treated with rVIII-SingleChain by approximately 45–50% [9]. The PK analysis was based on FVIII activity in plasma samples determined with the ChS assay as described previously [4].

Statistics

Efficacy analyses were conducted in patients who received at least one dose of rVIII-SingleChain as part of either on-demand treatment or routine prophylaxis. The ABR was calculated according to the following formula: (number of treated bleeds/efficacy evaluation period) $\times 365.25$. Descriptive statistics for the individual ABR/AsBR included the mean, standard deviation (SD), median and interquartile range. In addition, the number of bleeds per year and

the 95% confidence interval were estimated based on a Poisson model to account for variations in the follow-up period.

Safety was assessed in all patients exposed to rVIII-SingleChain. The primary safety endpoint was the cumulative incidence of inhibitor development. An exact two-sided 95% Clopper-Pearson confidence interval (or one-sided 97.5% upper confidence limit) was used for estimating the cumulative incidence of inhibitor formation.

Results

Study population

Of the 88 patients screened, 84 met the study eligibility criteria (Fig. 1). Patients were enrolled at 37 sites in 19 countries in Europe, the USA and the rest of the world. Countries contributing > 10% of subjects per age group were Thailand (in both age groups), France, the Philippines (0 to < 6 years group) and Turkey (≥ 6 to < 12 years group).

Characteristics and demographics of the 84 patients who received rVIII-SingleChain are displayed in Table 2. Thirty-nine study participants (0 to < 6 years, $n = 20$; ≥ 6 to < 12 years, $n = 19$) underwent a PK investigation. Investigators assigned patients to either a prophylaxis regimen (81 of 84) or to an on-demand regimen with rVIII-SingleChain (3 of 84, Fig. 1).

Overall, 65 of the 84 (77.4%) patients achieved ≥ 50 EDs to rVIII-SingleChain, with a similar proportion in each age group achieving ≥ 50 EDs (27 out of 35 [77.1%] in the 0 to < 6 years age group and 38 out of 49 [77.6%] in the ≥ 6 to < 12 years age group). Eight (9.5%) patients, all of whom were in the ≥ 6 to < 12 years age group, achieved ≥ 100 EDs. Study participants accumulated a total of 5239 EDs with rVIII-SingleChain, with a median follow-up period of 5.6 months.

PK of rVIII-SingleChain

PK parameters are summarized in Table 3. The analysis of the PK of a single intravenous (IV) dose of 50 IU kg $^{-1}$ rVIII-SingleChain included 39 patients aged 1 to 11 years. The FVIII activity profiles and the mean PK parameters were similar between the two age groups. Clearance was similar between age groups, with a mean clearance of 4.63 mL h $^{-1}$ kg $^{-1}$ in the ≥ 6 to < 12 years age group and 5.07 mL h $^{-1}$ kg $^{-1}$ in the 0 to < 6 years age group. Half-life was also consistent between the two age groups, with a mean half-life of 10.4 and 10.2 h for the 0 to < 6 years and ≥ 6 to < 12 years age groups, respectively.

rVIII-SingleChain in the control of bleeding events

A total of 347 bleeding events were treated with rVIII-SingleChain and assessed by the investigator. Of these 347 events, 132 occurred in the on-demand arm and 215 occurred in the 80 patients in the prophylaxis arm.

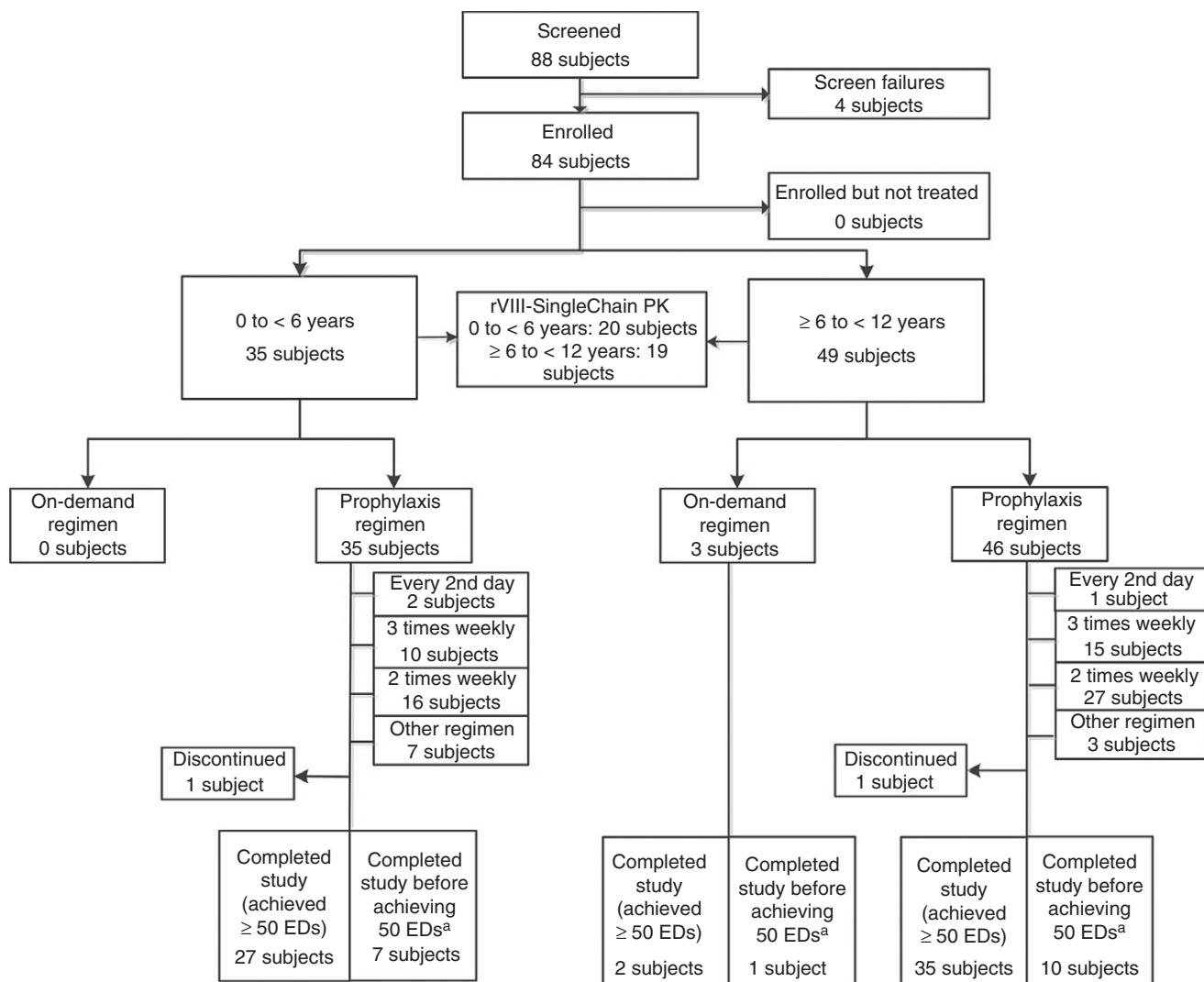


Fig. 1. Patient disposition in the study. EDs, exposure days; PK, pharmacokinetic(s); rVIII-SingleChain, recombinant single-chain factor VIII.

^aSubjects completed the study since the primary objective was achieved (i.e. 25 subjects achieving at least 50 EDs in the respective age cohort) and subjects could roll over to Extension Study 3001 in those centers in which the extension study was open for enrollment.

Hemostatic efficacy of rVIII-SingleChain was rated as excellent in 296 bleeds (85.3%), good in 38 bleeds (11%) and moderate in 12 bleeds (3.5%). One bleed was reported as poor or no response. Thus, treatment success (i.e. ratings of excellent or good) was 96.3% of all assessed bleeding events (Table 4). A total of 85.9% of the bleeding events were controlled with a single dose of rVIII-SingleChain, 9.8% with two doses and 4.3% with three or more doses. The median total dose used to treat a bleeding event was 27.6 IU kg⁻¹ (range 16–282 IU kg⁻¹) and the median dose per injection to treat a bleeding event was 27.3 IU kg⁻¹ (range 16–76 IU kg⁻¹).

rVIII-SingleChain in routine prophylaxis

Prior to enrollment in this study, 60 patients were treated with prophylaxis and 24 received on-demand therapy. A

comparison of previous and end-of-study treatment regimens for prophylaxis patients is displayed in Table 5. In the study, 43 (53%) of the 81 patients on prophylaxis were assigned to a two times weekly regimen (0 to < 6 years, n = 16; ≥ 6 to < 12 years, n = 27) and 25 (31%) to a three times weekly regimen (0 to < 6 years, n = 10; ≥ 6 to < 12 years, n = 15) (Fig. 1).

One patient (assigned to a three times weekly regimen) was excluded from the efficacy population because of a pre-existing low titer inhibitor that was not correctly reported at screening. Of the remaining 24 subjects in the three times weekly regimen, one was assigned a dose < 20 IU kg⁻¹ (19 IU kg⁻¹) and no subject was assigned a dose > 50 IU kg⁻¹. Of the 43 subjects in the two times weekly regimen, no one was assigned a dose < 20 IU kg⁻¹ and 40 subjects were assigned a dose between 20 and 50 IU kg⁻¹.

Table 2 Participant demographics and baseline characteristics

	< 6 years (n = 35)	≥ 6 to < 12 years (n = 49)	Total (n = 84)
Age (years)			
Median	4.0	9.0	7.0
Min, Max	1, 5	6, 11	1, 11
Q1, Q3	2, 5	7, 11	4, 9
Weight (kg)			
Median	16.00	32.00	25.00
Min, Max	10.0, 26.2	18.7, 87.5	10.0, 87.5
Q1, Q3	13.7, 19.6	26.3, 40.0	16.9, 34.9
BMI (kg m ⁻²)			
Median	15.63	17.60	16.80
Min, Max	13.4, 20.0	11.9, 29.6	11.9, 29.6
Q1, Q3	14.5, 17.0	16.1, 20.6	14.8, 18.9
Race (n [%])			
Asian	9 (25.7)	13 (26.5)	22 (26.2)
White	25 (71.4)	36 (73.5)	61 (72.6)
Other	1 (2.9)	0	1 (1.2)
Ethnicity (n [%])			
Hispanic or Latino	1 (2.9)	1 (2.0)	2 (2.4)
Not Hispanic or Latino	33 (94.3)	48 (98.0)	81 (96.4)
Not reported	1 (2.9)	0	1 (1.2)

BMI, body mass index; Max, maximum; Min, minimum. BMI is calculated as follows: BMI at screening = weight at screening (kg)/(height at screening [m])². Percentages are based on the number of subjects in the safety population.

Table 3 PK of rVIII-SingleChain in children aged 0–12 years following a single injection of 50 IU kg⁻¹

	Mean (CV%)	
	0 to < 6 years (n = 20)	≥ 6 to < 12 years (n = 19)
C _{max} , IU dL ⁻¹	80.2 (20.6)	83.5 (19.5)
IR, (IU dL ⁻¹)/(IU kg ⁻¹)	1.60 (21.1)	1.66 (19.7)
AUC _(0-last) IU·h dL ⁻¹	1010 (28.4)	1090 (26.4)
AUC _(0-∞) IU·h dL ⁻¹	1080 (31.0)	1170 (26.3)
t _{1/2} , h	10.4 (28.7)	10.2 (19.4)
CL, mL h ⁻¹ kg ⁻¹	5.07 (29.6)	4.63 (29.5)
V _{ss} , mL kg ⁻¹	71.0 (11.8)	67.1 (22.3)
MRT, h	12.4 (25.0)	12.3 (16.8)

AUC_{0-last}, area under the activity-time curve from zero (time of drug administration) to the time of last measurable (positive) activity; AUC_{0-∞}, area under the activity-time curve from zero (time of drug administration) to infinity with extrapolation of the terminal phase; CL, total plasma clearance; C_{max}, maximum observed concentration/activity; CV, coefficient of variation; IR, incremental recovery; MRT, mean residence time; n, total number of subjects; t_{1/2}, terminal elimination half-life of the compound; V_{ss}, volume of distribution at steady state. All parameters (except C_{max} and IR) are pre-dose uncorrected.

The median initially assigned rVIII-SingleChain dose was 32 IU kg⁻¹ on the three times weekly prophylaxis regimen and 35 IU kg⁻¹ on the two times weekly regimen. The median assigned dose at study end was 32 IU kg⁻¹ on the three times weekly prophylaxis

Table 4 Efficacy in the control of bleeding events

	n (%)
Bleeding events treated with rVIII-SingleChain	347
Bleeding events with investigator assessment	347 (100)
Efficacy rating	
Excellent	296 (85.3)
Good	38 (11.0)
Moderate	12 (3.5)
Poor/no response	1 (0.3)
Number of infusions required to treat	
1	298 (85.9)
2	34 (9.8)
3	8 (2.3)
> 3	7 (2.0)

Table 5 Comparison of pre- and end-of-study regimen in patients on prophylaxis

		Prophylaxis regimen at end of study (n = 48)			
		Every 2 days	3 times weekly	2 times weekly	Other
Prophylaxis regimen before enrollment in the study (n = 48)	Every 2 days	1	2	2	0
	3 times weekly	0	15	10	2
	2 times weekly	0	0	9	1
	Other	0	2	1	3

Sixty of the 84 subjects who enrolled in the study were treated with prophylaxis prior to study; data on the regimen prior to study are available for 48 of these 60 subjects. Dark gray shading indicates no change between regimen prior to enrollment and regimen in the study. Light gray shading indicates a decrease in injection frequency on study compared with prior to enrollment.

regimen and 35.5 IU kg⁻¹ on the two times weekly regimen. The percentage of prophylaxis subjects receiving at least one dose adjustment was similar between the two times weekly regimen (39.5%) and the three times weekly regimen (41.7%). The median annual consumption of rVIII-SingleChain on individualized prophylaxis across all prophylaxis regimens was 4109 IU kg⁻¹ (mean ± SD; 4312 IU kg⁻¹ ± 1491). A small number of patients shifted from the two times weekly (n = 6), every other day (n = 1) or once weekly (n = 1) regimens to a three times weekly regimen.

Across all prophylaxis regimens (n = 80), the observed median ABR was 3.69 (Q1, Q3: 0.00, 7.20) and the observed median AsBR was 0.00 (Q1, Q3: 0.00, 2.20). For joint bleeds, the observed median ABR was 1.62 (Q1, Q3: 0.0, 4.87). All ABRs (total, AsBR and joint bleed ABR) by regimen are listed in Table 6; ABRs by age group are displayed in Table 7 (total, AsBR and joint bleed ABR).

Investigators were allowed to adjust doses based on clinical bleeding phenotypes. Forty-nine patients on

Table 6 Efficacy in patients on prophylaxis.

	All regimens (n = 80*)	3 times weekly (n = 24*)	2 times weekly (n = 40†)
ABR			
Median (Q1, Q3)	3.69 (0.00, 7.20)	2.30 (0.0, 11.58)	4.37 (2.31, 7.24)
Estimated number of bleeds per year‡ (95% CI)	5.5 (4.8, 6.3)	5.8 (4.5, 7.4)	6.2 (5.2, 7.4)
AsBR			
Median (Q1, Q3)	0.0 (0.00, 2.20)	0.0 (0.00; 3.03)	0.0 (0.00, 2.08)
Estimated number of bleeds per year‡ (95% CI)	1.9 (1.5, 2.4)	1.8 (1.2, 2.9)	1.9 (1.4, 2.6)
Joint ABR			
Median (Q1, Q3)	1.62 (0.00, 4.87)	0.82 (0.00, 5.32)	1.93 (0.00, 4.61)
Estimated number of bleeds per year‡ (95% CI)	3.3 (2.7, 3.9)	3.1 (2.2, 4.4)	3.8 (3.1, 4.8)

ABR, annualized bleeding rate; AsBR, annualized spontaneous bleeding rate; Q1, Q3, interquartile range. *One subject has been excluded because of a pre-existing inhibitor. †Three subjects have been assigned doses outside the 15–50 IU kg⁻¹ range. ‡Estimated based on a Poisson model to account for variations in the follow-up period.

prophylaxis did not receive a dose adjustment during the study; these patients had an ABR of 2.73. Within this population, five children had ≥ 2 spontaneous bleeds within a 14-day period but the dose was not adjusted; these children had a median observed ABR of 6.94. In comparison, the 44 prophylaxis patients with no dose adjustment who did not have ≥ 2 spontaneous bleeds within a 14-day period had an ABR of 2.58. Patients with at least one dose adjustment had an ABR of 2.48 after dose adjustment compared with an ABR of 7.83 prior to dose adjustment.

Safety of rVIII-SingleChain

Immunogenicity Immunogenicity was assessed in all 84 patients exposed to rVIII-SingleChain. No patients developed an inhibitor during exposure to rVIII-SingleChain, including the 65 participants with ≥ 50 EDs, resulting in an inhibitor cumulative incidence of 0% (95% CI, 0.0–5.6%). One patient entered the study with a pre-existing low titer inhibitor (3.46 BU mL⁻¹) that was not correctly

reported at screening. Under an intensified prophylaxis regimen with rVIII-SingleChain (50 IU kg⁻¹ three times weekly) the patient became inhibitor negative after approximately 3 months and remained negative at the end of the study.

Ten participants entered the study with a positive test for non-inhibitory ADAs (i.e. anti-FVIII IgG and/or IgM antibodies) prior to dosing with rVIII-SingleChain. Ten other participants became positive for non-inhibitory ADAs during the study. In the 19 prophylaxis patients in the efficacy population (excluding the subject with the pre-existing inhibitor) who had a positive test for non-inhibitory ADAs at any time during the study, the median observed ABR (2.73) and the percentage of subjects with no bleeding episodes (31.6%) were similar to the values in the 80 subjects on prophylaxis overall. Four of the 10 subjects who tested positive for ADAs at screening participated in the initial PK investigation. The subjects with ADAs demonstrated similar PK parameters to the overall population in this study.

No patient had pre-existing anti-CHO antibodies or developed these during the study.

Table 7 Efficacy in patients on prophylaxis by age group

	0 to < 6 years (n = 35)	≥ 6 to < 12 years (n = 45*)
ABR		
Median (Q1, Q3)	2.12 (0.00, 4.54)	5.11 (2.52, 10.50)
Estimated number of bleeds per year† (95% CI)	3.0 (2.3, 4.0)	7.4 (6.3, 8.6)
AsBR		
Median (Q1, Q3)	0.00 (0.00, 1.46)	0.00 (0.00, 3.20)
Estimated number of bleeds per year† (95% CI)	0.9 (0.5, 1.5)	2.6 (2.0, 3.3)
Joint ABR		
Median (Q1, Q3)	0.00 (0.00, 1.89)	2.31 (0.00, 6.87)
Estimated number of bleeds per year† (95% CI)	1.1 (0.7, 1.8)	4.8 (4.0, 5.8)

ABR, annualized bleeding rate; AsBR, annualized spontaneous bleeding rate; Q1, Q3, interquartile range. *One subject has been excluded because of a pre-existing inhibitor. †Estimated based on a Poisson model to account for variations in the follow-up period.

Local tolerability rVIII-SingleChain was well tolerated. In the investigator assessment of local tolerability, 99.4% (307 of 309) of rVIII-SingleChain injections were assessed as ‘none’ (i.e. without erythema) and two patients had a total of two events of erythema categorized as ‘well defined’. With regards to itching, pain and heat severity, no reactions were reported except reactions assessed as ‘very slight’ in three patients. In the patient assessment of local tolerability, 99.4% (4747 of 4774) of injections were assessed as having no reaction. Of the 27 (0.56%) reactions that were reported, none were severe. No relevant differences were observed between age groups in either the investigator’s or the subject’s assessment of local tolerability.

Adverse events rVIII-SingleChain had an AE/serious AE (SAE) profile in line with the expected background

pathology for pediatric patients with severe hemophilia A, and as described for other products of the same class. In this study, 64 patients experienced a total of 183 treatment-emergent AEs, the majority ($n = 137$) being mild, 42 moderate and four severe in intensity. The most common AEs were nasopharyngitis, cough, headache and arthralgia. Only one AE (hypersensitivity) was considered by the investigator to be related to rVIII-SingleChain. The event was mild in intensity, non-serious and did not require a change in study medication. One AE resulted in withdrawal from the study (unrelated event of hip arthralgia, mild in intensity). Nine study participants experienced a total of 11 treatment-emergent SAEs: four patients in the 0 to < 6 years age group and five patients in the ≥ 6 to < 12 years age group (SAEs: hand fracture, laceration, traumatic rupture of the spleen, device occlusion, systemic inflammatory response syndrome, bacteremia, pneumonia, anemia and dyspepsia). No SAEs were considered by the investigator to be related to administration of rVIII-SingleChain. No thromboembolic events were observed.

Discussion

In this phase III pivotal study, a novel rFVIII in a single polypeptide chain configuration was evaluated in a large number of pediatric patients with severe hemophilia A. PK, efficacy and safety of rVIII-SingleChain for the treatment of bleeds and for routine prophylaxis were investigated. Hemostatic efficacy was rated as excellent or good in the vast majority (96.3%) of all bleeding episodes assessed by the investigator. In the children on prophylaxis, the median AsBR was 0.00 and the median ABR was 3.69, with more than 80% of children receiving rVIII-SingleChain either two or three times weekly at doses between 20 and 50 IU kg⁻¹. There were no cases of inhibitor development during the study and the nature and frequency of AEs were as expected for the study population.

Although rVIII-SingleChain demonstrated an improvement in PK parameters compared with a full-length non-modified rFVIII in adults and adolescents [4], the PK investigation in this pediatric study did not include a comparator to minimize blood draws. Comparing PK results across different studies, however, is problematic because most PK parameters are highly dependent on the method of analysis. In line with the observation that the clearance of FVIII generally decreases and $t_{1/2}$ increases from infancy to adulthood [10–13], the PK investigation of rVIII-SingleChain revealed that clearance was higher and $t_{1/2}$ was shorter in the pediatric population compared with adolescents and adults [4]. Based on these observed PK differences, almost all clinical trials mandate higher starting doses [14–16]. Recognizing that clearance varies significantly between individuals within the same age range [17], this study did not mandate specific pediatric dosing recommendations, but instead allowed for higher dosing and frequency depending on investigator discretion.

Recommended doses and schedules mirrored those of the rVIII-SingleChain adult and adolescent study [5].

The rVIII-SingleChain doses used to control bleeding episodes (median dose of 27.6 IU kg⁻¹) were within expectations for a rFVIII product [8]. Eighty-six per cent of all bleeds were effectively treated with just one infusion, and 96% with one or two infusions. rVIII-SingleChain in children showed similar or better efficacy in treating bleeds when compared with other recently approved rFVIII concentrates. Klukowska *et al.* reported efficacy ratings for on-demand treatment of breakthrough bleeding episodes with simoctocog alfa (Nuwiq[®]) as excellent or good for 82.4% of all bleeding episodes [18]. In a study in 71 children < 12 years of age treated with efmorococog alfa (Eloctate[®]), the reported treatment success was 89.4% of bleeding events, of which 93% were resolved with one or two injections [19]. Published data in children < 6 years of age treated with octocog alfa (Advate[®]) and children 0 to 11 years of age treated with turoctocog alfa (NovoEight[®]) reported treatment success rates of 93.8% and 92.1%, respectively [12,20].

As observed in the adult and adolescent study [5], the majority of subjects were assigned to a two times weekly (54%) or three times weekly regimen (30%) although a higher percentage of pediatric subjects as compared with the adult/adolescent study were assigned to a two times weekly regimen, possibly reflecting the desire for parents and children to minimize the number of injections per week. Children assigned to a two times weekly prophylaxis regimen were not prescribed higher doses per injection (median dose, 35 IU kg⁻¹) than subjects on a three times weekly regimen (median dose, 32 IU kg⁻¹) and rVIII-SingleChain consumption in pediatrics was nearly identical to that documented in the adults and adolescent study [5]. The median ABR and AsBR in patients treated three or two times weekly was low (2.30 and 0.00 / 4.37 and 0.00, respectively). Octocog alfa (Advate[®]) resulted in an ABR of 4.0 in subjects aged 0 to < 6 years and 5.3 in subjects aged > 7 to < 12 years on a three to four times weekly regimen [12,21], and turoctocog alfa (NovoEight[®]) resulted in an ABR of 3.02 in subjects on a three times weekly or every second day regimen [20]. Overall consumption was higher in both studies when compared with the consumption observed in this study; the Advate[®] study reported a median weekly consumption of 104 IU kg⁻¹ vs. 79 IU kg⁻¹ for rVIII-SingleChain and the NovoEight[®] study reported a mean monthly consumption of 462 IU kg⁻¹ vs. 359 IU kg⁻¹ for rVIII-SingleChain. Because the ABR for children on a three times weekly regimen with rVIII-SingleChain was lower than, and for children on a two times weekly regimen was similar to, those reported for Advate[®] and NovoEight[®], children treated with rVIII-SingleChain could achieve either comparable ABRs with fewer injections or a reduction in ABRs by nearly half with an unchanged infusion frequency.

Today, children with severe hemophilia are encouraged to exercise and take part in physical activities because good muscle strength supports joints and may reduce the frequency of bleeds, and studies have shown that participation in sports will have a direct impact on their overall health-related quality of life [22]. Prevention of bleeds in these active boys is challenging and requires meticulous attention from the prescribing physician and highly individualized therapeutic approaches. Although children in both the 0 to < 6 years and 6 to < 12 years age groups achieved AsBRs of 0.00, an increased ABR of 5.11 was observed in children aged 6 to < 12 years when compared with the ABR of 2.12 observed in children aged 0 to < 6 years. The increase in ABR was driven primarily by traumatic bleeds and thus is most likely to occur in a more active population. Therefore, in line with other recently approved products [19], more aggressive individualized doses and regimens should be considered for very active children who are more likely to experience traumatic bleeds. As such, this study demonstrates that individualized dosing can result in very low ABRs when investigators choose doses based on clinical bleeding phenotypes. The median ABR for subjects who did not have their dose or dosing schedule adjusted was low (2.58), although a subset of those subjects who had more than two spontaneous bleeding episodes in any 2-week interval but no dose adjustment had a significantly higher ABR (6.94). Similarly, the median ABR for subjects who had a dose adjustment was significantly higher (ABR = 7.83) prior to the dose adjustment when compared with the time post-adjustment (ABR = 2.48), highlighting the need for active monitoring of prophylaxis success in children with severe hemophilia A.

A low number of SAEs were documented in this study, none of which was considered related to rVIII-Single-Chain. No clinically relevant findings or safety concerns were observed in the vital signs and clinical laboratory data, and 99% of all injections were administered without any documented local reactions. The use of rVIII-Single-Chain for treatment of children with hemophilia A is also supported by the observation that there were no anaphylactic reactions, there was no inhibitor development under exposure to rVIII-SingleChain and no antibodies against CHO host cell proteins. As in studies of other recently approved rFVIII products, some children developed non-inhibitory ADAs ($n = 10$) during the study and 10 others entered the study with pre-existing non-inhibitory ADAs. It is important to mention that the ABR in patients with non-inhibitory ADAs was not higher than the overall ABR (2.73 vs. 3.69).

Overall, rVIII-SingleChain was highly effective in treating bleeding episodes in both age groups, and prophylactic treatment with rVIII-SingleChain resulted in a low ABR. rVIII-SingleChain offers many previously treated children the benefit of twice weekly prophylaxis with excellent tolerability and without the need to use a glyco-pegylated

or Fc fused product. Ongoing studies in previously untreated children will show if the increased binding to VWF will translate into a lower immunogenicity of rVIII-SingleChain compared with other rFVIII products. The differences in PK between adults and children should not necessarily mandate the use of higher doses on a population level. However, individualized doses and regimens should be considered for very active children who are more predisposed to traumatic bleeds. No inhibitor development was documented in the study and rVIII-SingleChain demonstrated a favorable safety profile.

Addendum

K. St. Ledger and A. Veldman contributed to the design of the study, the analysis and interpretation of data, and drafted the manuscript. N. Blackman contributed to the design of the study, analysis of data, and reviewed the manuscript. I. Pabinger contributed to the design of the study, interpretation of data, and reviewed the manuscript. O. Stasyshyn, G. Iosava, C. Djambas Khayat, J. Ong, K. Fischer, and F. Abdul Karim contributed to acquisition and interpretation of data, and reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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The AFFINITY Study Investigators are listed in the Appendix.

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Appendix

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