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Extension Study With rVIII-SingleChain in Previously Untreated Patients (PUPs) With Severe Haemophilia A

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ABSTRACT

Introduction: Clinical trials and real-world evidence have demonstrated the efficacy and safety of rVIII-SingleChain in previously treated patients with haemophilia A.

Aim: To investigate the safety and efficacy of rVIII-SingleChain in previously untreated patients (PUPs).

Methods: In an open-label, phase 3, extension study, PUPs with severe haemophilia A (FVIII <1%) received rVIII-SingleChain prophylactically or on-demand. The primary endpoints were incidence of high-titre (HT) inhibitor formation to FVIII, treatment success for major bleeding episodes and annualised spontaneous bleeding rate (AsBR).

Results: Twenty-four PUPs (median age 1 year [range 0–5]) were treated with rVIII-SingleChain; median time on study was 35.0 months (range 2.4–54.0). Overall, six PUPs developed a HT inhibitor (>5 BU/mL) and six developed a low-titre (LT) inhibitor (≤5 BU/mL). The median number of exposure days at inhibitor development was 10 (interquartile range [IQR] 5.0–14.0). Of 11 inhibitor-positive PUPs (five HT, six LT) who continued rVIII-SingleChain therapy, nine (81.8%; three HT, six LT) achieved inhibitor eradication (<0.6 BU/mL). Median time to eradication was 14.3 weeks (IQR 9.8–53.8). Seventeen treatment-emergent adverse events in 12 PUPs (50.0%) were related to rVIII-SingleChain, mainly inhibitor development (14/17 events). Treatment was successful (haemostatic efficacy rated excellent or good) for 290/315 bleeding events (92.1%). During prophylactic therapy, inhibitor-negative PUPs had a median (IQR) AsBR of 0.52 (0.00–4.99) and annualised bleeding rate of 1.98 (0.77–11.23).

Conclusion: RVIII-SingleChain demonstrated a satisfactory benefit:risk profile in PUPs, with a high treatment success rate and a low AsBR during prophylaxis, and was effective at eradicating inhibitors.

1 | Introduction

Haemophilia A is an X-linked bleeding disorder caused by a deficiency in coagulation factor VIII (FVIII) [1]. Patients with severe haemophilia experience spontaneous and post-traumatic bleeds; in rare cases, bleeding may be life-threatening [1]. Bleeding episodes can be controlled and prevented by FVIII replacement therapy. rVIII-SingleChain (AFSTYLA; CSL Behring) is a B-domain truncated recombinant FVIII (rFVIII) in which the heavy and light chains are covalently bonded into a single polypeptide chain, increasing the intrinsic molecular stability [2]. Relative to other rFVIII molecules, rVIII-SingleChain has a higher affinity to von Willebrand factor (vWF), which may prolong its half-life [2, 3]. Clinical trials and real-world evidence in previously treated patients (PTPs) have demonstrated the efficacy and tolerability of rVIII-SingleChain [4–10], which is approved for bleed treatment and prevention in patients with haemophilia A [11–13].

The main complication of FVIII replacement therapy is the development of anti-FVIII antibodies (inhibitors), which occur in ~30% of previously untreated patients (PUPs) [14, 15]. In the presence of inhibitors, patients become refractory to their FVIII treatment and may need to use bypassing agents (BPAs) [14, 16]. Patients who develop inhibitors may undergo immune tolerance induction (ITI) therapy, based on frequent administration of FVIII [1], with the aim of eradicating inhibitors and restoring responsiveness to FVIII replacement therapy [1, 17]. No inhibitors have been observed in PTPs in rVIII-SingleChain clinical trials.

As part of the AFFINITY clinical trial programme, the safety and efficacy of rVIII-SingleChain in PUPs was evaluated in an extension study. This included two substudies: one for inhibitor eradication and one for surgical procedures, also presented here.

2 | Methods

2.1 | Study Design

This phase 3, open-label, multicentre, extension study (NCT02172950) was part of the AFFINITY clinical trial programme, which enrolled both PTPs and PUPs. The long-term efficacy and safety of rVIII-SingleChain in PTPs was published previously [8]. For PUPs, inclusion criteria were severe haemophilia A (FVIII activity <1%), age <18 years and no prior exposure to any FVIII product. Individuals with a family history of FVIII inhibitors (first-degree relatives) or another known congenital or acquired coagulation disorder were excluded. Genetic analysis was conducted if *F8* gene mutation information was unavailable.

The study was conducted according to the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki 2008 [18, 19]. Ethical approval and individual informed consent were obtained before enrolment. An Independent Data Monitoring Committee oversaw safety [4].

PUPs who developed FVIII inhibitors could remain in the main study (if they had persistent low titres [≤5 BU/mL]) or join the inhibitor eradication (ITI) substudy (irrespective of the peak titre). Each investigator decided whether PUPs would be administered ITI therapy for up to 24 months. PUPs achieving inhibitor eradication could continue in the main study for 12 months; those not achieving eradication within 24 months or experiencing inhibitor relapse were withdrawn.

PUPs requiring surgery were eligible to participate in the surgery substudy, with rVIII-SingleChain administered before, during and after surgery. Participants could rejoin the main study after their procedure.

2.2 | Dosing

In the main study, PUPs were assigned by the investigator to prophylaxis or on-demand treatment with rVIII-SingleChain. The dose was based on World Federation of Hemophilia (WFH) guidelines and the PUP's bleeding phenotype, age and physical activity. Doses were adjusted throughout the study based on clinical judgment. Bleeding events could be treated with rVIII-SingleChain (and/or BPAs for inhibitor-positive PUPs).

Inhibitor-positive PUPs were assigned to ITI schedules based on clinical judgment. rVIII-SingleChain was given as ITI therapy at a low- (50 IU/kg, 3× weekly), intermediate- (100 IU/kg daily), or high-dose (200 IU/kg daily). Each PUPs dose could be decreased but not increased and bleeding events were treated with BPAs only.

2.3 | Antibodies/Inhibitors

Antibodies against rVIII-SingleChain and Chinese hamster ovary (CHO) cell proteins were assessed using a validated enzyme-linked immunosorbent assay (ELISA). Positive samples for CHO cell proteins were subject to a confirmatory assay using Surface Plasmon Resonance (SPR) technology. Confirmatory assays for antibodies against rVIII-SingleChain were not SPR-based. Non-inhibitory anti-drug antibodies (ADA) screening test results were followed by confirmatory ADA immunoglobulin (Ig) G and M tests. The result was positive if both the screening and the confirmatory tests were positive. FVIII-specific antibodies were classified according to isotype, subclass and FVIII binding region. Inhibitory FVIII antibodies were determined using the Nijmegen-modified Bethesda assay [20]. PUPs with two consecutive central laboratory (CL) results of ≥0.6 BU/mL were diagnosed as inhibitor-positive. Inhibitor eradication was defined as CL results for two consecutive visits <0.6 BU/mL and confirmed by incremental recovery evaluation; if inhibitor eradication was achieved, patients could continue on prophylaxis. PUPs were tested for antibodies, including inhibitors, at each visit. Scheduled visits were every month until 25 exposure days (EDs) were achieved and then every 3 months until the end of study.

2.4 | Safety Endpoints

The primary safety endpoint was the incidence of high titre (HT) inhibitor formation to FVIII in PUPs with ≥50 EDs to

rVIII-SingleChain. Incidence rates for FVIII inhibitors were stratified by peak titre (HT, >5 BU/mL; low titre [LT], ≤5 BU/mL). Secondary safety endpoints included the percentages of PUPs who developed antibodies against rVIII-SingleChain and CHO cell proteins. Treatment-emergent adverse events (TEAEs) were recorded and classified by system organ class and preferred term using the Medical Dictionary for Regulatory Activities (Version 21.1).

2.5 | Efficacy Endpoints

The primary efficacy endpoints were treatment success for major bleeding episodes and the annualised spontaneous bleeding rate (AsBR) during prophylaxis and on-demand treatment. Major bleeding episodes were defined as those for which a subject was required to seek treatment from the treating physician if it threatened life or loss of limb (e.g., intracranial haemorrhage, gastrointestinal bleeding, and severe bleeding). All other bleeding episodes were classified as minor. Treatment success was defined as a rating of 'excellent' or 'good' on the investigator's clinical assessment of haemostatic efficacy, based on a 4-point scale (excellent, good, moderate or poor/no response) [4].

Secondary efficacy endpoints included treatment success for non-major bleeding episodes, number of injections to achieve haemostasis, annualised bleeding rates (ABRs) and consumption of rVIII-SingleChain.

2.6 | Exploratory Endpoints

Exploratory endpoints included an ITI substudy that investigated inhibitor eradication rate, time to eradication and consumption of rVIII-SingleChain in PUPs who developed an inhibitor to rVIII-SingleChain, and a surgery substudy that evaluated treatment efficacy using a 4-point scale (excellent, good, moderate or poor/no response).

2.7 | Statistical Analysis

Continuous data were summarised using descriptive statistics and categorical data were summarised using frequency counts and percentages. Two-sided, 95% confidence intervals (CI) were calculated for critical parameters, but no statistical comparisons were performed. The analysis population comprised all enrolled PUPs who received at least one dose of rVIII-SingleChain.

AsBR was calculated in inhibitor-positive and inhibitor-negative PUPs as follows:

([Number of spontaneous bleeding episodes]/[observed treatment period of interest]) × 365.25

AsBR was summarised by prophylaxis or on-demand treatment using descriptive statistics. The number of spontaneous bleeds per year and associated 95% CI were based on a Poisson distribution.

TABLE 1 | Demographic and baseline characteristics of the study population. Data shown are median (range) or *n* (%) unless otherwise indicated.

Characteristic	Value (*N* = 24)
Age (years)	1.0 (0–5)
Country	
South Africa	8 (33.3%)
Italy	4 (16.7%)
Lebanon	3 (12.5%)
Netherlands	3 (12.5%)
Portugal	3 (12.5%)
Malaysia	2 (8.3%)
USA	1 (4.2%)
Race	
White	15 (62.5%)
Black or African American	7 (29.2%)
Asian	2 (8.3%)
Body weight (kg)	9.9 (3.8–20.0)
FVIII gene mutation[a]	
Intron 22 inversion	8 (33.3%)
Frameshift mutation[b]	4 (16.7%)
Nonsense mutation	3 (12.5%)
Large deletion[b]	2 (8.3%)
Missense mutation	2 (8.3%)
Point mutation	1 (4.2%)
Other	4 (16.7%)

[a]In general, mutations causing the absence or dysfunctional production of FVIII tend to carry a higher risk for inhibitor formation: intron 22 inversion and large deletions are high-risk mutations; missense mutations can vary in risk, but some lead to misfolded or dysfunctional FVIII and may provoke an immune response; and frameshift mutations carry a high risk if they lead to premature stop codons or non-functional FVIII.
[b]One frameshift mutation and one large deletion were obtained from the FVIII database (www.factorviii-db.org).

3 | Results

3.1 | Study Population

Twenty-four PUPs were enrolled from seven countries (Table 1); 62.5% were white and the median age at enrolment was 1 year (range 0–5). Nineteen PUPs completed the study (reasons for the five withdrawals were: physician decision [*n* = 3], adverse event [*n* = 1], other [*n* = 1]).

All PUPs had at least one risk factor for inhibitor development (e.g., black race, *F8* gene mutation) [21, 22]. Genetic mutations associated with risk of inhibitor development were found, including intron 22 inversions (*n* = 8, 33.3%), large deletions (*n* = 2, 8.3%), nonsense mutations (*n* = 3, 12.5%), missense mutations (*n* = 2, 8.3%), frameshift mutation (*n* = 4, 16.7%), point mutation (*n* = 1, 4.2%) and other (*n* = 4, 16.7%) [Table 1].

Twelve PUPs were initially assigned to prophylaxis and 12 to on-demand treatment. Of the latter, 11 switched to prophylaxis for reasons including increased activity, growth, physician decision and inhibitor development; these individuals contributed to both the on-demand and prophylaxis data.

All 24 enrolled PUPs were exposed to rVIII-SingleChain. Overall, 5914 injections of rVIII-SingleChain were administered, at a median dose per injection of 50.0 IU/kg (IQR 40.6–57.5). Median time on study was 35.0 months (range 2.4–54.0) and the median number of EDs per PUP was 230.0 (IQR 142.0–323.0); >50 EDs were recorded for 21 PUPs (87.5%), two PUPs (8.3%) had <10 ED and one had >10 to 25 EDs (4.2%). In the on-demand group, median total dose per bleeding episode requiring treatment was 31.4 IU/kg (range 22–150), and most bleeding episodes resolved with one or two infusions (76.5% and 14.7%, respectively). In the prophylaxis group, total weekly median (IQR) doses of 32.0 IU/kg (25.5–40.7), 80.0 IU/kg (49.3–88.6), and 150.0 IU/kg (148.0–170.5) were administered in the PUPs receiving 1× ($n = 22$), 2× ($n = 15$) and 3× ($n = 12$) weekly treatment, respectively. Of the PUPs who did not develop an inhibitor, the majority were treated 1× ($n = 5$) or 2× weekly ($n = 4$).

3.2 | Safety

3.2.1 | Inhibitor Development

Twelve PUPs (50.0%) were diagnosed with a FVIII inhibitor (six LT, six HT) (Table 2). At the time of inhibitor development, the median number of EDs was 10 (range 4.0–23.0). Median (range) peak inhibitor titre before initiation of ITI treatment was 1.6 BU/mL (0.7–3.3) and 34.3 BU/mL (5.9–140.0) in the LT and HT groups, respectively (Table 2). Out of the 22 subjects with at least 50 EDs or who developed an inhibitor within 50 EDs, six subjects (27.3%) were diagnosed with a HT anti-FVIII inhibitor. No subjects developed an anti-FVIII inhibitor after 50 EDs.

FVIII-specific IgG was detected in all inhibitor-positive patients, comprising of IgG subclasses 1, 3 and 4. All patients with LT inhibitors only had one FVIII-specific IgG, all HT inhibitor patients had at least two IgG subclasses specific for FVIII. Interestingly, only the two patients with HT and without inhibitor eradication had three subclasses (IgG1, 3 and 4) specific for FVIII. Eleven patients had antibodies that recognised epitopes on both the heavy and light chain of FVIII, and one patient had a LT inhibitor that only recognised epitopes on the light chain of FVIII.

3.2.2 | Inhibitor Eradication

Of the 12 inhibitor-positive PUPs, 11 received ITI treatment (six LT, five HT); six of whom enrolled in the exploratory ITI substudy (see *ITI substudy*). During ITI treatment, the median (IQR) number of EDs was 88.0 (30.0–189.0); the median (IQR) total ITI dose was 160.2 (149.3–202.7) IU/kg/week. During ITI treatment, the median (IQR) AsBR and ABR in inhibitor-positive PUPs were 0.47 (0.0–2.8) and 0.47 (0.0–3.6), respectively. These bleeding rates were comparable to those of inhibitor-negative PUPs on prophylactic treatment (Table 3).

Overall, 9/11 inhibitor-positive PUPs treated with rVIII-SingleChain (81.8%) had successful inhibitor eradication (<0.6 BU/mL); one remained inhibitor-positive throughout the study and one was withdrawn before completion of treatment. Inhibitor eradication was achieved after a median (IQR) of 14.3 weeks (9.6–53.6); 53.6 weeks (51.1–64.4) in PUPs with HT inhibitors ($n = 3$) and 11.2 weeks (8.1–26.0) in those with LT inhibitors ($n = 6$).

In inhibitor-positive PUPs, 19 bleeding events were treated with rVIII-SingleChain; the treatment success rate was 68.4% (95% CI 35.5%–89.5%) (Table 4). Poor/no response was observed for three bleeding events, necessitating additional treatment with BPAs (proximal inhibitor titres before the bleeding events were 28.5, 5.9 and 2.6 BU/mL).

All 11 inhibitor-positive PUPs experienced at least one TEAE during ITI treatment. Five treatment-emergent serious adverse events (TESAEs) were reported by four PUPs (36.4%), of which two were related to rVIII-SingleChain.

3.2.3 | Adverse Events

Overall, 320 TEAEs were reported (Table 5). Seventeen TEAEs in 12 PUPs (50.0%) were related to rVIII-SingleChain, the majority of which comprised inhibitor development ($n = 14$, 82.4%). There were 21 TESAEs in 14 PUPs (58.3%); 12 of these comprised inhibitor development. Thirteen TESAEs (12 that comprised inhibitor development plus one haemorrhage) were related to rVIII-SingleChain.

3.2.4 | Non-Inhibitory Anti-Drug Antibodies (ADAs)

No PUPs developed antibodies against CHO cell proteins. At screening, five PUPs (20.8%) were positive and 19 (79.2%) were negative for rVIII-SingleChain non-inhibitory ADAs; seven of whom remained negative throughout the study. Overall, 12 PUPs (50.0%) became positive for ADAs during the study, of whom eight (66.7%) developed an inhibitor (two LT, six HT). Of the other four patients who developed an inhibitor (four LT), two were positive for ADAs at screening and remained positive during the study, and two were ADA negative at screening and the confirmatory tests were not performed during the study and so the presence of an ADA could not be confirmed. No allergic reactions or lack of efficacy were associated with non-inhibitory ADA positivity.

3.3 | Efficacy

Of 315 bleeding events, 312 were treated with rVIII-SingleChain alone and three with rVIII-SingleChain and a BPA (Table S1). One bleeding episode (spontaneous haemarthrosis of the right knee) was classified as major, 60 were minor and 254 were undefined. Treatment for the major bleeding event comprised a single infusion of rVIII-SingleChain (36 IU/kg); haemostatic efficacy was rated as excellent. Treatment for minor bleeding events was successful (rated as excellent or good) for 54 events (90.0%).

TABLE 2 | Details of inhibitor development in the study population.

Subject	Regimen prior to inhibitor treatment	ITI regimen	ED of initial positive-inhibitor result	Peak titre BU/mL	Duration inhibitor-positive (months)	Current status (current titre BU/mL)	Mutation details
High titre ($N = 6$)							
1[a]	30 IU/kg, 2× week	50 IU/kg, 3× week	13	53.1	25.5[b]	Eradicated (<0.6)	Intron 22 inversion
2[a]	40 IU/kg, 1× week	50 IU/kg, 3× week[c]	4	120.0	13.1	Positive (63.0)	Large deletion of ≤1 exons
3[a]	50 IU/kg, 2× week	50 IU/kg, 3× week	14	140.0	25.8	Positive (99.0)	Deletion of exon 5 to 9
4	23 IU/kg, 2× week	42 IU/kg, 3× week	23	5.9	13.4	Eradicated (<0.6)	Intron 22 inversion
5	30 IU/kg, 1× week	Withdrawn[d]	5	12.9	1.2	Withdrawn[d]	C.453_459DELINSGGAAGG, P.SER153GLUFS*9 in exon 4
6[a]	30 IU/kg, 2× week	200 IU/kg, daily	5	15.5	7.9	Eradicated (<0.6)	Intron 22 inversion
Median (range)	—	—	9 (4.0, 23.0)	34.3 (5.9, 140.0)	13.4[e] (7.9, 25.5)	—	—
Low titre ($N = 6$)							
7	38 IU/kg, 1× week	50 IU/kg, 3× week	11	2.6	15.7	Eradicated (<0.6)	Intron 22 inversion
8[a]	32 IU/kg, 1× week	57 IU/kg, 3× week	8	3.3	4.4	Eradicated (<0.6)	C.2830_2831DELAA, P.LYS944VALFS*6
9	21 IU/kg, 1× week	58 IU/kg, 3× week	19	0.8	4.3	Eradicated (<0.6)	C209_212DE 1 P.(PHE70*)
10[a]	25 IU/kg, 1× week	54 IU/kg, 3× week	9	1.3	2.9	Eradicated (<0.6)	C.2102_2106DELTGGAA, P.MET701LYSFS*27 in exon 13
11	25 IU/kg, 1× week	50 IU/kg, 2× week	23	0.7	2.5	Eradicated (<0.6)	Intron 22 inversion
12	30 IU/kg, 1× week	30 IU/kg, 1× week	5	1.9	6.0	Eradicated (<0.6)	Intron 22 inversion
Median (range)	—	—	10 (5.0, 23.0)	1.6 (0.7, 3.3)	4.3 (2.5, 15.7)	—	—
All ($N = 11$)[d]							
Median (range)	—	—	10 (4.0, 23.0)	4.6 (0.7, 140.0)	6.0[e] (2.5, 25.5)	—	—

Abbreviations: BU, Bethesda units; ED, exposure days; ITI, immune tolerance induction; PUP, previously untreated patient.

[a]Patients who participated in the ITI substudy.

[b]Treatment with rVIII-SingleChain was withheld for 1 year to allow the inhibitor titre to decrease and to allow participation in the ITI substudy.

[c]PUP subsequently switched to a regimen of 100 IU/kg every other day.

[d]PUP was withdrawn per protocol, due to diagnosis of a high-titre inhibitor before implementation of the ITI substudy.

[e]Excluding PUPs who remained inhibitor-positive at the end of the study.

TABLE 3 | Summary of bleeding rates by inhibitor status and rVIII-SingleChain regimen.

	All bleeding events		While inhibitor-negative		While inhibitor-positive	
Regimen	**On-demand**	**Prophylaxis**	**On-demand**	**Prophylaxis**	**On-demand**	**Prophylaxis**
Number of evaluable PUPs[a]	10	23	10	21	0	11
AsBR, median (IQR)	1.2 (0.0–4.0)	0.9 (0.0–5.0)	1.2 (0.0–4.0)	0.5 (0.0–5.0)	—	0.5 (0.0–2.8)
AsBR, mean (SD)	1.9 (2.3)	4.0 (6.4)	1.9 (2.3)	4.3 (7.0)	—	2.1 (3.3)
ABR, median (IQR)	3.8 (0.8–9.0)	1.8 (0.8–11.2)	3.8 (0.8–9.0)	2 (0.8–11.2)	—	0.5 (0.0–3.6)
ABR, mean (SD)	5.1 (5.3)	5.9 (7.7)	5.1 (5.3)	6.6 (8.3)	—	2.4 (3.3)
AjBR, median (IQR)	1.2 (0.0–3.6)	1.4 (0.3–2.3)	1.2 (0.0–3.6)	1.5 (0.4–3.1)	—	0.0 (0.0–0.8)
AjBR, mean (SD)	1.6 (1.8)	1.4 (1.3)	1.6 (1.8)	1.7 (1.5)	—	0.5 (1.0)

Abbreviations: ABR, annualised bleeding rate; AjBR, annualised joint bleeding rate; AsBR, annualised spontaneous bleeding rate; IQR, interquartile range; PUP, previously untreated patient; SD, standard deviation.
[a]PUPs required a minimum of 8 weeks of exposure per inhibitor status and per regimen (on-demand or prophylaxis) to be evaluable.

TABLE 4 | Summary of haemostatic efficacy of rVIII-SingleChain according to bleeding type and inhibitor status.

	All bleeding events[a]		Bleeding events[a] while inhibitor-negative		Bleeding events[a] while inhibitor-positive	
Haemostatic efficacy	***n***	**%**	***n***	**%**	***n***	**%**
Number of PUPs with bleeds[a]	21	—	21	—	6	—
Total bleeds[a]	315	—	296	—	19	—
Excellent	278	88.3	268	90.5	10	52.6
Good	12	3.8	9	3.0	3	15.8
Moderate	21	6.7	18	6.1	3	15.8
Poor/no response	3	1.0	0	0.0	3	15.8
Missing	1	0.3	1	0.3	0	0.0

Abbreviation: PUP, previously untreated patient.
[a]Only bleeding events treated with rVIII-SingleChain are included.

Haemostatic efficacy was rated as excellent for 278 events (88.3%) and good for 12 (3.8%) (Table 4). Treatment success rate was 92.1% (95% CI 87.0%–95.3%) overall, 93.6% (95% CI 89.1%–96.3%) in the absence of inhibitors and 68.4% (95% CI 35.5%–89.5%) in the presence of inhibitors. Haemostasis was achieved with 1–2 infusions of rVIII-SingleChain in 280 events (88.9%); 17 events (5.4%) required three infusions and 11 events (3.5%) required >3 infusions (Figure 1).

Median (IQR) AsBR and ABR were 1.2 (0.0–4.0) and 3.8 (0.8–9.0) in the on-demand group, and 0.9 (0.0–5.0) and 1.8 (0.8–11.2) in the prophylaxis group, respectively (Table 3). Median (IQR) AjBR was 1.2 (0.0–3.6) and 1.4 (0.3–2.3) for the on-demand and prophylaxis regimens, respectively.

3.4 | ITI Substudy

Of the 12 PUPs who developed inhibitors (whole ITI population), six participated in the planned ITI substudy, one patient was excluded prior to ITI substudy initiation and five continued prophylaxis in the main study (Table 2). The investigators decided whether patients would be administered ITI therapy. At the time of ITI substudy enrolment, four PUPs had HT inhibitors and two had LT inhibitors. Overall, 5/6 participants received low-dose ITI (50 IU/kg, 3× weekly) and one received a high dose (200 IU/kg daily). Overall, 4/6 participants achieved inhibitor eradication (<0.6 BU/mL).

3.5 | Surgery Substudy

Three surgeries (two circumcisions and one port placement surgery) were conducted in three PUPs. All surgeries were performed successfully and the efficacy of rVIII-SingleChain was rated as excellent in all procedures. No adverse events were reported.

4 | Discussion

This is the first trial to evaluate the safety and efficacy of rVIII-SingleChain in PUPs with haemophilia A. Overall, treatment was well tolerated and treatment success for bleeding events

TABLE 5 | Summary of treatment-emergent adverse events.

	Number of events	Total *n* (%) PUPs
Any TEAE	320	24 (100.0)
Any TEAE of special interest	15	10 (41.7)
TEAE severity		
Mild	208	24 (100.0)
Moderate	93	16 (66.7)
Severe	17	9 (37.5)
No grading	2	2 (8.3)
Most frequent TEAEs		
Pyrexia	44	15 (62.5)
Upper respiratory tract infection	18	7 (29.2)
Nasopharyngitis	15	9 (37.5)
Cough	13	6 (25.0)
Rhinitis	10	6 (25.0)
Any TEAE related to rVIII-SingleChain	17	12 (50.0)
Inhibitor development[a]	14	12 (100.0)
Fatigue	1	1 (4.2)
Pyrexia	1	1 (4.2)
Haemorrhage	1	1 (4.2)
Any TESAE	21	14 (58.3)
Any TESAE leading to withdrawal	1	1 (4.2)
Any TESAE related to rVIII-SingleChain	13	12 (50.0)

Abbreviations: FVIII, factor VIII; PUP, previously untreated patient; TEAE, treatment-emergent adverse event; TESAE, treatment-emergent serious adverse event.

[a]Inhibitor development includes the preferred terms 'anti-factor VIII antibody positive', 'inhibiting antibodies positive' and 'factor VIII inhibition'.

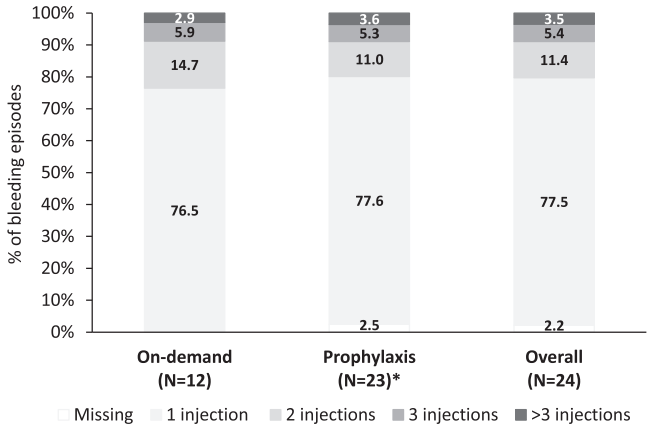

FIGURE 1 | Number of rVIII-SingleChain injections required to achieve haemostasis. *Prophylaxis subject total included 12 subjects assigned to prophylaxis plus 11 of 12 on-demand subjects who later switched to prophylaxis, per the investigator's decision.

was 92%, comparable with results from phase 3 clinical trials of rVIII-SingleChain in PTPs (94%–96%) [4, 7].

The inhibitor development rate for PUPs treated with rVIII-SingleChain was 50% (95% CI 29%–71%). It is generally accepted that inhibitors develop in 25%–35% of PUPs receiving FVIII therapy, but higher rates (up to 50%) have been reported [15, 24, 26-31]. Patients treated with rFVIII had a higher incidence of inhibitors (44.5% [95% CI, 34.7–54.3]) than those treated with plasma-derived FVIII (pdFVIII) containing vWF (26.8% [95% CI, 18.4–35.2]) in the Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) trial [15]; however, some clinicians have methodological concerns about the study, including differences in ethnicity compared with their patient population, the rFVIII products used, non-applicability of the SIPPET findings to extended half-life (EHL) rFVIII products, a lower assay cutoff for inhibitor diagnosis and a predominance of patients with null mutations that may have affected the outcomes [32]. In a recent analysis of the European HAemophilia Safety Surveillance (EUHASS) project and Canadian Haemophilia Surveillance System (CHESS), inhibitor rates in PUPs were much lower with standard half-life rFVIII (27% [95% CI, 24–30]) and rEHL FVIII (22% [95% CI, 12–36]) than observed in SIPPET, although still slightly higher than with pdFVIII (20% [95% CI, 14–26]) [33]. The proportion of inhibitors that were HT in this study was 50%, which is slightly lower than reported in other studies of PUPs with haemophilia A (range: 55%–79%), possibly due to the small sample size, while the rate of HT inhibitors in our study was 25%, which is similar to previously reported rates (19%–28%) [15, 24, 26, 28, 30, 31]. It is possible that our study employed a more frequent inhibitor testing regimen (every month until 25 EDs) compared with other studies or the real-world setting. Our study showed a high incidence of LT inhibitors that achieved inhibitor eradication (<0.6 BU/mL) within a median time of 14 weeks. These may not be detected in a real-world setting as the LT inhibitor may still protect patients from bleeding and, as a consequence, these patients would not be tested for the presence of an inhibitor.

Several risk factors have been identified for the development of FVIII inhibitors [34]. Genetic risk factors include the severity of haemophilia A, family history of inhibitors and high-risk *F8* gene mutations including large deletions, nonsense mutations and intron 22 inversions. Treatment-related risk factors include early/intensive FVIII treatment and immunologic/inflammatory/infectious events (e.g., surgery, illness) [14, 15, 31, 35]. Alongside having severe haemophilia A, all participants in our study had at least one risk factor for inhibitor development and 8/12 PUPs who developed inhibitors had a high-risk mutation. At the time of inhibitor development, all PUPs were on a prophylaxis regimen. Overall, 8/12 PUPs who developed non-inhibitory ADAs also developed FVIII inhibitors; six had HT and two had LT inhibitors.

The median number of EDs at the time of inhibitor development was 10.0 (range 5.0–23.0). This aligns with observations that most inhibitors develop during the first 50 EDs [27]. Early

detection of inhibitors is essential to ensure adequate treatment of bleeding episodes and an appropriate approach to eradicating the antibodies. Frequent inhibitor testing (at least every five to ten EDs or every 3 months) has been recommended until 50 EDs are completed [36].

In patients with inhibitors, effective treatment of bleeding episodes is challenging. HT inhibitors neutralise FVIII, curtailing response to the principal treatment for bleeding in haemophilia A. BPAs are used to treat acute bleeding in patients with inhibitors. Still, these have inconsistent haemostatic efficacy and may increase disease-related morbidity [17]. Whereas efficient prophylaxis is feasible in inhibitor patients using emicizumab, inhibitor eradication is the primary goal to allow safe and effective treatment of bleeds and surgery [37–39]. ITI is the only established means of achieving this goal, whereby high/frequent doses of FVIII concentrate are administered until the immune system can tolerate FVIII [40]. Success rates of 74%–87% have been reported with ITI [16, 41]. Here, inhibitor eradication was achieved in 9/11 (82%) PUPs who received rVIII-SingleChain, mainly with a low-dose regimen (≤50 IU/kg, 3× weekly). Furthermore, the number of bleeds was lower in the period after inhibitor development than in the prophylaxis period without inhibitor. The lower bleeding rates are likely due to the more frequent dosing of patients undergoing ITI, as reported in a study investigating determinants of bleeding before and during ITI in boys with severe haemophilia A and HT inhibitors [42]; furthermore, patients with a LT inhibitor may have a similar or lower bleeding rate due to FVIII still preventing spontaneous bleeds.

Among inhibitor-negative PUPs receiving prophylactic regimens, we found the median ABR and AsBR rates to be low (1.98 and 0.52, respectively). The treatment success rate and ABR were similar to other rFVIII products in PUPs [23–25]. For example, the overall Poisson mean estimated ABR for turoctocog alfa for patients on prophylaxis was 4.26 bleeds/patient/year (95% CI, 3.34−5.44) [24]. In all surgeries, rVIII-SingleChain demonstrated excellent haemostatic efficacy without any safety concerns.

There were several limitations in our study. The small sample size and lack of sample size calculation limited statistical robustness, as reflected by relatively large CIs. When it was designed (in 2015), the study was intended to include at least 50 PUPs to meet the European Medicines Agency (EMA) regulatory requirements. However, in 2018, the EMA removed the obligation for clinical trials in PUPs due to the limited availability of data and recruitment for our study was stopped earlier than planned [43]. The EMA recommended data collection from patient registries rather than from small clinical trials that may not be fully representative of the real-world use of the drug [43]. Currently, there is limited data on rVIII-SingleChain in PUPs available from the PedNet Haemophilia Registry, however, there might be more patients in the future. The use of negative binomial modelling to calculate ABR in this study was pre-specified in the study protocol; however, the use of other statistical methods (e.g., Poisson distribution) may have yielded different results.

Exclusion of PUPs who had a first-degree relative with inhibitors may have lowered the observed inhibitor rate. Conversely, all participants had at least one known risk factor for inhibitor development. Finally, the ITI substudy included only a limited number of PUPs and there was variability in rVIII-SingleChain dosing.

5 | Conclusion

rVIII-SingleChain demonstrated a satisfactory benefit:risk profile in PUPs. The incidence of HT inhibitors was consistent with rates observed with other rFVIII products. Most PUPs who developed inhibitors continued to receive rVIII-SingleChain and low doses were sufficient to achieve eradication in most cases.

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Ethics Statement

The study was conducted according to the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki 2008. Ethical approval and individual informed consent were obtained before enrolment. An Independent Data Monitoring Committee oversaw safety.

Conflicts of Interest

Authors conflict of Interest are stated under disclosure

Data Availability Statement

CSL will only consider requests to share Individual Patient Data (IPD) that are received from systematic review groups or bona-fide researchers. CSL will not process or act on IPD requests until 12 months after article publication on a public website. An IPD request will not be considered by CSL unless the proposed research question seeks to answer a significant

and unknown medical science or patient care question. Applicable country-specific privacy and other laws and regulations will be considered and may prevent sharing of IPD. Requests for use of the IPD will be reviewed by an internal CSL review committee. If the request is approved, and the researcher agrees to the applicable terms and conditions in a data sharing agreement, IPD that has been appropriately anonymised will be made available. Supporting documents including study protocol and Statistical Analysis Plan will also be provided. For information on the process and requirements for submitting a voluntary data sharing request for IPD, please contact CSL at clinicaltrials@cslbehring.com.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.