Recombinant B-domain-deleted porcine sequence factor VIII (r-pFVIII) for the treatment of bleeding in patients with congenital haemophilia A and inhibitors

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Introduction: Development of inhibitors to human FVIII (hFVIII) significantly complicates the control of bleeding events in patients with haemophilia A. Aim: This prospective, multicentre, open-label, non-comparative, Phase II study evaluated the haemostatic activity of a recombinant B-domain-deleted porcine FVIII (r-pFVIII), in the treatment of non-life/non-limb-threatening bleeding in individuals with haemophilia A and FVIII inhibitors. Methods: Acute bleeding episodes in patients with pFVIII inhibitor titres <0.8 BU mL⁻¹ were treated with 50 U kg⁻¹ body weight r-pFVIII. Those with pFVIII inhibitor titres >0.8 BU mL⁻¹ received an initial calculated r-pFVIII loading dose followed by 50 U kg⁻¹ treatment dose. Treatment continued at 6-hourly intervals until bleeding was determined, controlled or till a maximum of eight doses was reached. Results: All 25 bleeding episodes in nine patients (mean age: 23.7 years; range: 14 – 34 years) were controlled successfully with eight or fewer injections of r-pFVIII. The median time from bleeding onset to the administration of r-pFVIII was 5.7 h (range: 1.5 – 20.0 h). Twenty of the bleeding episodes (80%) were controlled with one treatment dose of r-pFVIII (with or without a loading dose, median dose: 200.8 U kg⁻¹; range: 50–576 U kg⁻¹) regardless of pFVIII level. r-pFVIII was well tolerated and no treatment-emergent serious adverse events were considered by the investigator to be related to r-pFVIII administration. Conclusion: The results suggest that FVIII replacement therapy with r-pFVIII could be a viable alternative to bypassing agents for the treatment of bleeding episodes in individuals with haemophilia A and FVIII inhibitors.

Keywords: factor VIII, factor VIII deficiency, haemophilia A, haemophilia A, congenital, recombinant proteins
Thirty years of clinical experience with plasma-derived porcine FVIII (Hyate:C) demonstrated low cross-reactivity with human FVIII (hFVIII) inhibitors and established clinical efficacy of porcine FVIII to control bleeding in individuals with HA and inhibitors [9–15]. OBIZUR (OBI-1/BAX801; Baxalta Inc., Deerfield, IL, USA) is a recombinant B-domain-deleted porcine sequence factor VIII (r-pFVIII), with low cross-reactivity to hFVIII inhibitors. r-pFVIII is sufficiently similar to hFVIII to be haemostatically active, but sufficiently different in structure to render it less susceptible to inactivation by inhibitory antibodies [16]. In preclinical proof-of-concept studies, r-pFVIII produced dose-dependent control of bleeding in two HA animal models [17–19]. In mice, r-pFVIII had comparable immunogenicity to Hyate:C [18,20]. In dogs, r-pFVIII demonstrated greater recovery and similar half-life to Hyate:C [21]. In a Phase I study in non-bleeding individuals with congenital HA with inhibitors, a single r-pFVIII dose had higher bioavailability than Hyate:C. These data from preclinical models suggest that r-pFVIII is haemostatically active [18–20]. The aim of this study was to evaluate the ability of r-pFVIII to control bleeding in patients with severe HA and inhibitors.

Methods

Study design

This was a Phase II, prospective, multicentre, open-label, non-comparative study (ClinicalTrials.gov identifier: NCT00245245). The primary objective was to evaluate the haemostatic activity of r-pFVIII in the treatment of non-life/non-limb-threatening bleeding in individuals with HA and inhibitors. The secondary objectives of the study included evaluating the safety and immunogenicity of r-pFVIII.

The study protocol was approved by the respective human research ethics committees at all participating sites. All study patients (or their parents/guardians) provided signed, informed consent prior to study participation. The study was conducted in accordance with the Declaration of Helsinki [22], International Conference of Harmonization and Good Clinical Practice (GCP) Guidelines [23].

Study patients

This multicentre study was conducted at 14 sites across Canada, USA, Russia and South Africa. Individuals ≥12 years (≥18 years in Russian centres) with a diagnosis of congenital HA with inhibitors to hFVIII, and pFVIII antibody titre of ≤20 Bethesda Units (BU mL−1) at screening were eligible for enrolment. Patients must have had a current treatment plan for an acute bleeding episode that required the use of inhibitor bypassing treatment and uncomplicated joint or soft tissue bleed, or other non-life-threatening or non-limb-threatening bleeding episode. Patients were excluded if they had received hFVIII within 7 days prior to screening, an aPCC within 7 days prior to treatment with r-pFVIII, or rFVIIa within 3 days prior to screening or treatment with r-pFVIII. Patients with life- or limb-threatening bleeding episode (intracranial haemorrhage or severe head trauma, penetrating wound of the abdomen or thorax, intra-abdominal or gastrointestinal haemorrhage, iliopsoas haemorrhage, throat or neck haemorrhage), any condition potentially compromising vital functions or significant liver disease (defined as 5× the upper limit of normal for transaminases, oesophageal varices or hepatomegaly) or renal disease (creatinine >2.5 mg L−1) were excluded. Following enrolment, medical records were reviewed to determine the most recently recorded hFVIII inhibitor and pFVIII inhibitor levels (BU mL−1) and the length of time they had been present.

Treatment regimen

Patients with an acute bleeding episode with pFVIII inhibitor titre <0.8 BU mL−1 were given an r-pFVIII treatment dose of 50 U kg−1. Those with inhibitor titre >0.8 BU mL−1 (lower limit of detection) [24] received an initial r-pFVIII loading dose calculated as follows:

\[
\text{Plasma volume} \times \text{body weight} \times \frac{80 \text{ mL kg}^{-1}}{1-\text{haematocrit}} \times \text{pFVIII inhibitor titre (BU/mL)}.
\]

Immediately after completion of the loading dose, patients received 50 U kg−1 r-pFVIII, the likely dose to achieve a therapeutic blood level between 30% and 125%. The aim of the loading dose was to neutralize circulating pFVIII antibodies prior to induction of haemostasis. All r-pFVIII doses for the first bleeding episode (including the loading dose, if required) were infused at a rate of 1 mL min−1 (500 U min−1). The infusion rate was increased to 2 mL min−1 if no infusion-related events followed the first infusion. Five and half to 6 h following each r-pFVIII administration, the investigator and the patient assessed bleeding response to treatment using the patient’s signs and symptoms as either controlled or not controlled. If the bleeding episode was not controlled, further doses were given at 6-hourly intervals up to a maximum of eight doses or 1000 U kg−1 in a 24-h period. The first, second and third treatment doses were 50 U kg−1, the fourth, fifth and sixth doses were 100 U kg−1 and the seventh and eighth doses were 150 U kg−1. The increasing successive doses aimed to overcome anamnestic response to baseline antibodies. If antibody levels rose over time, higher doses would be required to reach target FVIII levels. Treatment was considered unsuccessful if the bleeding episode was not controlled after eight doses,
or if the investigator deemed r-pFVIII ineffective before all eight doses were given.

Treatment of subsequent bleeding episodes with r-pFVIII was permitted for patients with a previous bleeding event that was successfully managed with r-pFVIII without drug-related serious adverse events (SAE). In this study, bleeds not treated with r-pFVIII during study participation were recorded as adverse events. The administration of r-pFVIII for treatment of the first two bleeding episodes was given under medical supervision at each patient’s treatment centre. Patients who were successfully treated with OBI-1 for at least two bleeding episodes were able to manage subsequent bleeding episodes in a home care programme. If r-pFVIII was administered outside of the centre, the patient was required to contact the centre within 6 h of r-pFVIII injection to assess control of the bleeding episode.

Study evaluations

For each patient treated with r-pFVIII, all bleeding episodes and subsequent treatments were recorded and analysed. The time from onset of symptoms to initiation-of-treatment and the location and type of bleeding episode were recorded. Haemostatic control was determined by clinical assessment of signs and symptoms by both the investigator and the patient. The number of treatment doses and dose given was also recorded.

Safety assessments included vital signs prior to treatment and 0.5 h following each treatment dose for bleeding episodes one and two; a clinical assessment 5.5–6.0 h after each dose; haematology (complete blood count, differential, platelet count), biochemistry (alanine transaminase, aspartate transaminase) and coagulation screening tests [prothrombin time (PT), activated partial thromboplastin time APTT]; assessments prior to treatment, platelet counts 0.5 and 5.5–6.0 h after treatment, and haematology assessments at 14 and 28 days posttreatment, and thereafter every 16 weeks after the first injection bleeding episode one. Monitoring ceased after 24 weeks after the last injection of r-pFVIII. All patients were monitored closely throughout the study.

All inhibitor titres were measured at a central laboratory using the Nijmegen modification of the Bethesda assay [25]. Specific pFVIII inhibitor titres and hFVIII inhibitor titres were determined from blood collected at screening, prior to the first dose, day 14 and day 28 for all bleeding episodes. If the pFVIII inhibitor titre was >0.8 BU mL⁻¹ at day 28, further titres were obtained every 4 weeks until two consecutive levels were ≤0.8 BU.

Antibody titres against BHK proteins were measured on blood samples collected at screening, day 28 after each r-pFVIII dose, then at the 6-month visit, every 16 weeks thereafter and end-of-study (EOS). Antibody titres were measured by enzyme-linked immunosorbent assay (ELISA) and quantified as a measurement of optical density (OD) (Covance Method ELISA-0192R2; Covance Laboratories Inc., Chantilly, VA, USA); an OD >0.08 was defined as the limit of positive detection (LOPD) for anti-BHK antibody titres.

Plasma levels of FVIII were determined prior to and 0.5 h after each dose. FVIII levels were measured by two independent methods: one-stage coagulation assay (OSCA) and chromogenic assay (Esoterix Inc., Austin, TX, USA). All clinical assessments were made without knowledge of the level of FVIII attained with the injection being assessed for purposes of objective blinding; with the exception of the FVIII levels determined as part of pharmacokinetic assessments, all investigators, study personnel and patients were blinded to the results of the FVIII measurements throughout the study.

Statistical methods

Descriptive analyses were performed on all data collected. Continuous variables were summarized using mean, standard deviation, median, minimum and maximum.

Results

Patients and disposition

Twenty-eight individuals were screened at 14 study sites in Canada, the United States of America, Russia and South Africa; four patients did not meet the eligibility criteria, leaving 24 individuals eligible for participation in the study (Fig. 1). Nine patients [the intent-to-treat (ITT) population] experienced at least one bleed and received r-pFVIII for a total of 25 eligible bleeding events. Seven patients in the ITT population completed the study, and two discontinued prematurely (Fig. 1). The reasons for early discontinuation were withdrawn consent (n = 1) after one full treatment episode and discontinuation of study by the sponsor (n = 1), due to patient preference. While the
discontinuation of study withdrawal was following DSMC review, it was not related to AEs.

Demography

The mean age in the ITT population was 23.7 years [Range (SD): 14–34 (6.3) years]. All nine patients were male (three black, six white, of whom one identified as Hispanic, eight non-Hispanic). Eight patients had severe HA (<1% FVIII activity) and one (patient 9) had moderate HA (1–5% FVIII activity) with inhibitors requiring bypassing agent treatment. The peak hFVIII inhibitor titre in eight patients was >10.1 BU mL⁻¹ at least 1 year prior to enrolment; the highest historical hFVIII inhibitor titre was ≤5 BU mL⁻¹ in the remaining patient. At the time of first bleed in the study, hFVIII inhibitor titres were in the range <0.8–15.7 BU mL⁻¹, while pFVIII inhibitor titres were <0.8–6.2 BU mL⁻¹ (historic pFVIII inhibitor levels were not available for all patients).

Haemostatic control and dosing with r-pFVIII

All 25 bleeding episodes experienced by the nine patients were controlled successfully with ≤8 r-pFVIII doses; the median number of injections per bleeding episode was 1 (range: 1–8; Table 1). Twenty of 25 (80%) bleeding episodes were controlled with one r-pFVIII dose (with or without loading dose; median dose: 200.8 U kg⁻¹; range: 50–576 U kg⁻¹). For all bleeding episodes, median initial dose (with or without a loading dose) was 159 U kg⁻¹ (range: 50–576 U kg⁻¹), with a loading dose used in 17/25 (68%) bleeds (Table 1). The median total dose of r-pFVIII required to control a bleeding episode was 224.1 U kg⁻¹ (range: 50.0–1066.4 U kg⁻¹). Four patients were treated for one bleeding episode and five were treated for more than one (Table 1). The median time from bleeding onset to administration of r-pFVIII was 5.7 h (range: 1.5–20.0 h).

Safety

The DSMC did not express safety concerns that warranted study termination. Patients received a total dose range of 50–1066.4 U kg⁻¹ for bleeding episodes. Of the 61 AEs reported, 18 (in seven patients) were considered TEAEs. Three TEAEs in two patients were considered possibly related to the study drug. One patient had two distinct episodes of a mild infusion reaction (mild itching/pruritus) during the first bleeding episode that resolved with treatment (diphenhydramine). The reaction did not recur after re-treatment with r-pFVIII for a second bleed. One patient had an increase in alanine aminotransferase and aspartate aminotransferase levels after one dose of r-pFVIII to control a single bleed. Of note, this patient was hepatitis C positive at screening. This event was conservatively reported as a TEAE, although the timing of the blood draw in relation to r-pFVIII administration was not recorded. Three patients reported three serious TEAEs, all of which were bleeding events occurring during the study period at different loci to the initial bleed. None of these events were considered by the investigator to be related to r-pFVIII. No patient discontinued the study due to an AE. No patient produced detectable levels of antibodies against BHK at any time during the study (all OD readings were below the LOPD). All other safety assessments conducted were considered to be within normal limits for the study population.

Immunogenicity and FVIII results

For all study bleeding episodes, hFVIII and pFVIII inhibitor titres at the time of bleed were within <0.8–15.7 BU mL⁻¹ (median: 4.6 BU mL⁻¹; mean: 5.7 BU mL⁻¹) and <0.8–40.4 BU mL⁻¹ (median: 1.7 BU mL⁻¹; mean: 6.0 BU mL⁻¹) respectively. Two patients had lower hFVIII inhibitor titres at EOS compared with pretreatment levels, five had higher titres and there was no change in two (<0.8 BU mL⁻¹; Table 1). Three patients who had pFVIII inhibitor titres <0.8 BU mL⁻¹ at the time of the first bleed developed de novo anti-pFVIII antibodies (Table 1). There were five patients with positive pFVIII inhibitor titres at baseline who subsequently ended the study with higher anti-pFVIII titres. In those who received >1 r-pFVIII dose, spikes in pFVIII inhibitor titres diminished over time (Fig. 2). The safety and efficacy of r-pFVIII in these patients did not appear to be
**Table 1.** Haemostatic efficacy of OBI-1 for the treatment of non-life/non-limb-threatening bleeds: number of doses required to control bleeding, immunogenic response [porcine factor VIII (pFVIII) inhibitor and human factor VIII (hFVIII) inhibitor] and peak FVIII levels during the study [one-stage coagulation assay (OSCA) and chromogenic assay].

| Pt | Bleeding episode | Location and type of bleed* | Symptom onset to treatment (h) | OBI-1 administration | Total injections [n] [dose (U kg⁻¹)] | pFVIII inhibitor titre (BU mL⁻¹)† | hFVIII inhibitor titre (BU mL⁻¹)† | Peak FVIII level (%) | OSCA | Chromogenic assay |
|----|------------------|-----------------------------|-------------------------------|----------------------|------------------------------------|-----------------------------------|-----------------------------------|---------------------|------|------------------|
| 1  | 1                | Left ankle haemarthrosis    | 4.3                           | 50                   | 3 [150]                            | 0.8                               | 5.8                               | 2.4                 | 25.2 | 60               |
| 2  | 1                | Left ankle haemarthrosis    | 9.55                          | 74 LD + 50           | 3 [224]                            | 0.8                               | 40.4                              | 5.1                 | 25.2 | 83               |
| 3  | 1                | Right shoulder haemarthrosis| 4.78                          | 50                   | 1 [50]                             | 40.4                              |                                    |                     |                  |                  |
| 2  | 2                | Right elbow haemarthrosis   | 12.75                         | 50                   | 1 [50]                             |                                    |                                    |                     |                  |                  |
| 3  | 1                | Foreskin haematoma          | 4                             | 109 LD + 50          | 1 [159]                            | 1.9                               |                                    |                     |                  |                  |
| 4  | 1                | Circumcision suture site haematoma | 3.3   | 91 LD + 50 | 1 [141]                            |                                    |                                    |                     |                  |                  |
| 5  | 1                | Circumcision suture site haematoma | 7     | 91 LD + 50 | 1 [141]                            |                                    |                                    |                     |                  |                  |
| 6  | Ulva bleed       | 11.5                         | 517 LD + 50                   | 1 [567]               | 11.6                               |                                    |                                    |                     |                  |                  |
| 7  | Gum bleed        | 15                            | 526 LD + 50                   | 1 [576]               | 18.4                               |                                    | 6.6                               |                     |                  |                  |
| 8  | Left forearm/right knee muscle bleed | 11     | 526 LD + 50 | 1 [576]             | 30 & 23.2                          |                                    |                                    |                     |                  |                  |
| 4  | 1                | Left knee haemarthrosis     | 5.25                          | 65 LD + 50           | 1 [115]                            | 1.1                               | 20.2                              | 8.5                 | 3.8 & 0.9 | 8.4            |
| 2  | 2                | Lower lip bleed             | 4.5                           | 525 LD + 50          | 4 [775]                            | ND                                |                                    |                     |                  |                  |
| 3  | 3                | Right elbow haemarthrosis   | 5                             | 254 LD + 50          | 1 [304]                            | 6.4                               |                                    |                     |                  |                  |
| 4  | 4                | Left elbow haemarthrosis    | 3                             | 254 LD + 50          | 1 [304]                            | 7.9                               |                                    |                     |                  |                  |
| 5  | 1                | Gum bleed                   | 3.83                          | 193 LD + 50          | 1 [243]                            | 6.2                               | 149.4                             | 22                  | 14 & 9.8 | 667.4 | 51.9          |
| 6  | 1                | Right knee/abdominal muscle bleed | 20    | 50       | 2 [100]                            | <0.8                              | 13.6                              | <0.8                | 2.3                 | 19.4  | 1.4            |
| 2  | 2                | Right knee haemarthrosis    | 6.08                          | 50                   | 1 [50]                             | 1.4                               |                                    |                     |                  |                  |
| 3  | 3                | Right middle finger haemarthrosis | 1.75   | 316 LD + 50 | 1 [366]                            | 1.5                               | <0.8                              |                     |                    |                  |
| 7  | 1                | Left wrist haemarthrosis    | 18.08                         | 50                   | 1 [50]                             | 4.2                               | 143.4                             | 6.8                 | 5.3 & 5.5 | 14 & 1.7 | 1.7     |
| 2  | 2                | Right bicep muscle bleed    | 14.33                         | 316 LD + 50          | 8 [1066]                           | 3.5                               |                                    |                     |                  |                  |
| 3  | 3                | Left ankle haemarthrosis    | 15                            | 518 LD + 50          | 1 [568]                            | 7.3                               |                                    |                     |                  |                  |
| 4  | 4                | Left flank muscle bleed     | 1.5                           | 470 LD + 50          | 1 [520]                            | <0.8                              |                                    |                     |                  |                  |
| 8  | 1                | Left knee/inner left side muscle bleed | NK   | 264 LD + 50 | 1 [314]                            | 5.6                               | 34                                | 13.2                 | 15.7                 | 45.7  | 16.7          |
| 9  | 1                | Left elbow haemarthrosis    | 2.33                          | 50                   | 1 [50]                             | <0.8                              | <0.8                              | <0.8                | <0.8              | <0.8   | <0.8       |

LD, Loading dose; NK, not known; ND, no data (result below 1% detection limit); pFVIII, porcine factor VIII; hFVIII, human factor VIII; BU, Bethesda Unit; OSCA, one-stage coagulation assay.

*Classification of bleeding episodes based on criteria described in EMEA guideline on Core SPC for Human Plasma Derived and Recombinant Coagulation Factor VIII Products Rev.1 19 July 2007.

†If two values were obtained, both values were reported.
affected by the pFVIII inhibitor titre, with no increase in AEs, including bleeding episodes, reported in those with the highest titres.

There was a median increase in FVIII plasma levels 30 min following the initial treatment dose (with or without a loading dose): median increase 16% (<0.5–427%) with the OSCA and 17% (1–248%) with the chromogenic assay (Table 1). The increase in FVIII levels was variable both among patients and during bleeding episodes in the same patient. Useful FVIII recoveries (>40%) were obtained following one of three infusions given when pFVIII inhibitor titres were 10–20 BU mL$^{-1}$, 3/3 infusions when titres were ≥2.5–10 BU mL$^{-1}$ and 10/22 infusions when the titres were <2.5 BU mL$^{-1}$.

FVIII levels did not correlate with pretreatment pFVIII inhibitor titres (Fig. 3). The considerable variability in pharmacokinetic parameters calculated for each patient precluded the determination of a full pharmacokinetic profile for r-pFVIII. However, mean

Fig. 2. Immunogenic responses [porcine factor VIII (pFVIII) and human FVIII (hFVIII) inhibitor levels] in patients who received more than one treatment dose of OBI-1. Arrows denote bleeding episodes.
FVIII recovery values in response to r-pFVIII were substantially higher in patients without measurable pFVIII inhibitor titres at the time of first bleed (prior to treatment with r-pFVIII), than in those with measurable pFVIII inhibitors (Table 2). All bleeding episodes were successfully controlled regardless of the pretreatment pFVIII inhibitor titres or the FVIII recovery values after treatment with r-pFVIII.

**Discussion**

Overall, r-pFVIII appeared effective and well tolerated in patients with HA and anti-hFVIII inhibitors. After reviewing the safety and efficacy data on nine patients and 25 bleeds, the DSMC concluded that further data would not modify the conclusion that r-pFVIII was effective in establishing haemostasis. As a result of this assessment, the sponsor decided to close the study early.

Of the 61 AEs reported on or after the first day of treatment, only 18 were categorized as treatment emergent. Porcine FVIII inhibitor titres varied among study patients and bleeding events, and were not related to the dose and total r-pFVIII exposure. For example, patient 5 had the third lowest cumulative dose (243 U kg⁻¹) in 1 day but the highest increase in anti-pFVIII titre. The ability of r-pFVIII to control a bleed was not affected by the pFVIII inhibitor level, as has been previously observed by Brettler et al. [9].

In patients with congenital HA and FVIII inhibitors, r-pFVIII treatment effectively controlled all non-life/ non-limb-threatening bleeding episodes with a median of 1 dose, even in the presence of high hFVIII inhibitor titres. This finding establishes the potential role of r-pFVIII as a useful alternative treatment for bleeds in individuals with HA and inhibitors.

All 25 bleeding episodes were controlled and of these, haemostasis was achieved in 20 patients with only one treatment dose of r-pFVIII. As such, r-pFVIII may be attractive alternative to currently available bypassing agents, which, despite their relative but variable efficacy [26,27], do not provide the same invariable haemostasis observed in patients without inhibitors following FVIII replacement [4]. Notably, r-pFVIII is not a bypassing agent; it exerts its effect by restoring haemostatic activity in patients with HA in the presence of hFVIII inhibitors. Its efficacy is achieved in part from the lack of cross-reactivity between pFVIII and hFVIII inhibitors. In this context, r-pFVIII meets the need for a FVIII replacement therapy for patients with HA and inhibitors [27]. Similar to the use of FVIII replacement therapy in patients without inhibitors, FVIII activity can be objectively monitored using both one-stage clotting and chromogenic assays, can guide dosing decisions and act as a surrogate marker for safety and efficacy using validated FVIII assays. In contrast, currently available bypassing agents do not have a validated assay that correlates with clinical efficacy; consequently, safety and efficacy monitoring rely solely on subjective clinical assessments. Interassay variability was as expected when comparing the FVIII levels obtained with one-stage clotting and chromogenic assays. Overall, chromogenic assay tended to give lower values than the one-stage assay.

**Table 2.** PK parameters 30 min after administration of OBI-1 to treat non-life/non-limb-threatening bleeds (first bleed only).

| pFVIII inhibitor titre at time of first bleed (prior to treatment) | Parameter | OSCA | Chromogenic assay |
|---------------------------------------------------------------|----------|------|-------------------|
| <0.8 BU mL⁻¹ (n = 4)                                          | Cmax/D  ([U dL⁻¹]/[U kg⁻¹]) | 1.77 ± 1.22 | 0.95 ± 0.47 |
|                                                              | Tmax [range] (h) | 0.25 (0.25–0.50) | 0.5 (0.25–3.00) |
|                                                              | AUC 0–6 h/D | 5.67 ± 2.7 | 3.82 ± 1.50 |
| >0.8 BU mL⁻¹ (n = 4*)                                         | Cmax/D  ([U dL⁻¹]/[U kg⁻¹]) | 0.08 ± 0.09 | 0.06 ± 0.06 |
|                                                              | Tmax [range] (h) | 0.25 (0.25–0.50) | 0.63 (0.25–3.00) |
|                                                              | AUC 0–6 h/D | 0.24 ± 0.31 | 0.18 ± 0.20 |

pFVIII, porcine factor VIII; OSCA, one-stage coagulation assay; BU, Bethesda Unit.

*One patient with pFVIII inhibitor levels >0.8 BU mL⁻¹ at the time of the first bleed had no detectable FVIII level.
clotting assay, a phenomenon described in the prescribing information for r-pFVIII and in agreement with a recent field study determining assay variability for measuring r-pFVIII activity [28].

A loading dose was used during treatment of 17 bleeding episodes. The protocol specified a complex calculation of the loading dose in this study that is impractical in a clinical setting, as an acute bleed requires immediate treatment that cannot be delayed while the laboratory assays required for this calculation are completed (the pFVIII inhibitor assay takes at least 2 h). A more practical approach is to administer a fixed first dose of r-pFVIII that can be titrated based on clinical response and plasma FVIII levels. Using this strategy, bleeding was controlled in 80% of patients in this study with one dose of r-pFVIII at a median dose level of 200.8 U kg⁻¹, even in the presence of levels of hFVIII inhibitors (0.8–15.7 BU mL⁻¹ at the time of first r-pFVIII treatment). Consequently, an initial 200 U kg⁻¹ fixed dose of r-pFVIII was selected for use in future studies.

Although this was a small sample size study and data were not available for all parameters across all patients, available data provide valuable evidence relating to the use of r-pFVIII in non-life/non-limb-threatening bleeding episodes in individuals with HA and inhibitors. The results also corroborate evidence of clinical safety and efficacy seen in subjects with acquired HA [29]. Future studies that would further the clinical development programme of r-pFVIII in individuals with HA and inhibitors include a phase III surgery study that is currently recruiting patients as of this writing.

In summary, treatment with r-pFVIII provided satisfactory haemostasis, controlling all bleeding episodes in individuals with HA and inhibitors, even in the presence of high hFVIII or pFVIII inhibitor levels, and was well tolerated. The results from this study suggest that FVIII replacement therapy with r-pFVIII could be a viable alternative to bypassing agents for the treatment of bleeding episodes in individuals with HA and FVIII inhibitors regardless of pFVIII inhibitor titres. In addition, treatment with r-pFVIII allows for monitoring of FVIII levels to supplement clinical assessment, which is not achievable with currently available bypassing agents.

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Author contributions

J. Mahlangu executed the study, participated in data collection, analysis, interpretation, writing and critical evaluation of the manuscript and approved its submission for publication. T. A. Andreeva, D. E. Macfarlane, C. Walsh, N. S. Key participated in the data collection, and contributed to the writing and critical evaluation of the manuscript and approved its submission for publication.

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Disclosure of conflict of interests

D. Macfarlane received compensation from Octagen and Inspiration for performing the study, and has acted as consult to Baxalta, Bayer and CSL Behring. N. Key is the recipient of an investigator-initiated research grant from Baxalta Inc., and has acted as a consultant for CSL Behring, Bayer, Novo Nordisk and Alynlam. J. Mahlangu has received research funding from Bayer, Biogen, CSL Behring, Inspiration, Octagen, Novo Nordisk, Roche and has acted as consultant for Amgen, Bayer, Baxalta, Biogen, Genentech, Novo Nordisk, T. A. Andreeva and C Walsh declare no conflicts of interest.

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